Amylin: Pharmacology, Physiology, and Clinical Potential

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Abstract——**Amylin is a pancreatic β-cell hormone that produces effects in several different organ systems. Here, we review the literature in rodents and in humans on amylin research since its discovery as a hormone about 25 years ago. Amylin is a 37-amino-acid peptide that activates its specific receptors, which are multisubunit G protein–coupled receptors resulting from the coexpression of a core receptor protein with receptor activity–modifying proteins, resulting in multiple receptor subtypes. Amylin's major role is as a glucoregulatory hormone, and it is an important regulator of energy metabolism in health and disease. Other amylin actions have also been reported, such as on the cardiovascular system or on bone. Amylin acts principally in the circumventricular organs of the central nervous system and functionally interacts with other metabolically active hormones such as cholecystokinin, leptin, and estradiol. The amylin-based peptide, pramlintide, is used clinically to treat type 1 and type 2 diabetes. Clinical studies in obesity have shown that amylin agonists could also be useful for weight loss, especially in combination with other agents.**

ABBREVIATIONS: 5CNAC-sCT, *N*-(5-chlorosalicyloyl)-8-aminocaprylicacid; AD, Alzheimer's disease; AP, area postrema; BAT, brown adipose tissue; BMI, body mass index; CCK, cholecystokinin; CeA, central amygdala; CGRP, calcitonin gene–related peptide; DBH, dopamine-β-hydroxylase; DIO, diet-induced obese; ERK1/2, extracellular signal–regulated kinase 1 and 2; GLP-1, glucagon-like peptide 1; GPCR, G protein–coupled receptor; LPB, lateral parabrachial nucleus; MC, melanocortin; NTS, nucleus of the solitary tract; OVX, ovariectomized; PEG, polyethylene glycol; RAMP, receptor activity–modifying protein; SFO, subfornical organ; STAT3, signal activator of transcription 3; U0126, 1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene; UCP, uncoupling protein; VBS, visible burrow system; VMH, ventromedial hypothalamus; VTA, ventral tegmental area.

I. Introduction

Since its discovery in 1986, the hormone known as amylin has been the subject of extensive research that has ultimately translated into the approved diabetes drug pramlintide acetate (Symlin; AstraZeneca, Wilmington, DE). A timeline that outlines some of the major discoveries and important achievements in this field is presented in Fig. 1. Pramlintide is an analog of amylin, which has been developed as a therapeutic for the treatment of both type 1 and type 2 diabetes mellitus. Although the currently approved therapeutic indications for amylin agonists are in the diabetes space, the bulk of research into amylin biology over the past decade relates to its actions as a satiation hormone. This review covers historical aspects of amylin discovery, receptors, and physiology. In particular, we focus on amylin's well characterized effects on eating and weight regulation, with coverage of supporting pharmacology studies in preclinical and clinical studies. The prospect of combination therapies using amylin agonists is also covered in depth. The well established and emerging actions of amylin are summarized in Fig. 2 to provide an overview of its physiologic functions.

II. Amylin

A. Amylin and Related Peptides

Amylin, also known as islet/insulinoma amyloid polypeptide or diabetes-associated peptide, is a 37-amino-acid secreted hormone (Fig. 3), derived from an 89-amino-acid precursor (Westermark et al., 2011). The processing of the prohormone to yield amylin occurs via carboxypeptidase E and prohormone convertases 1/3 and 2 (Westermark et al., 2011). The gene encoding this peptide is found on chromosome 12 in humans (12p12.1). Two major post-translational modifications are required for full bioactivity; these are a C-terminal amide (Tyr37) and a disulfide bond formed between the two cysteine residues found at positions 2 and 7 (Fig. 3) (Young et al., 1996b). In terms of amino acid sequence, amylin is most closely related to calcitonin gene–related peptide (CGRP). CGRP also shares a similarly positioned disulfide bond and an

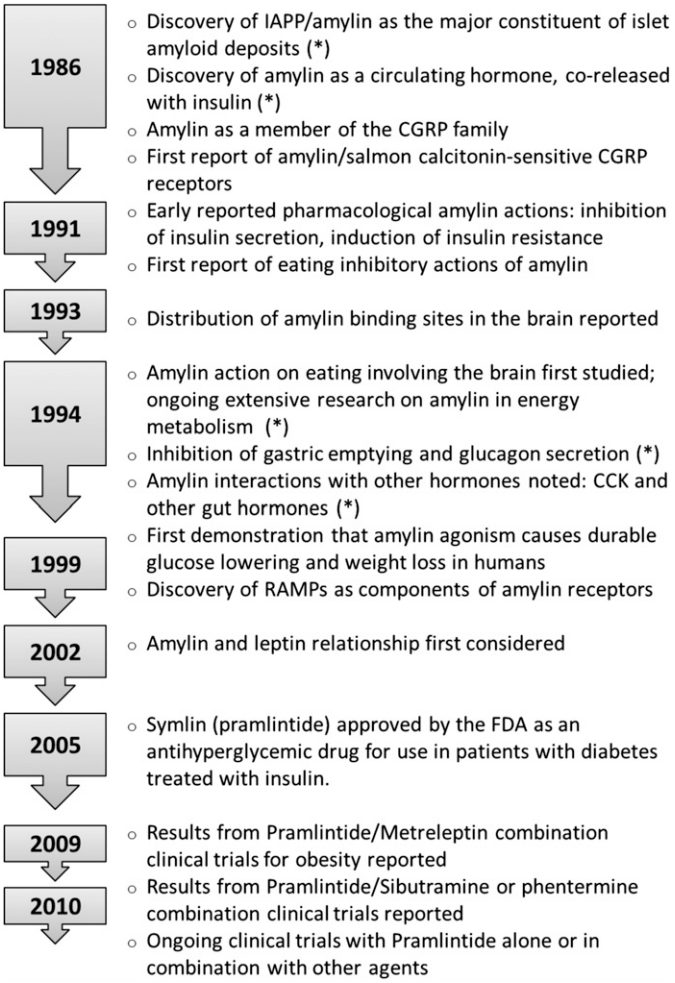

Fig. 1. A timeline of major discoveries and achievements in the field of amylin biology. Effects where convincing evidence for a physiologically or pathophysiologically relevant role of amylin exists are marked with an asterisk.

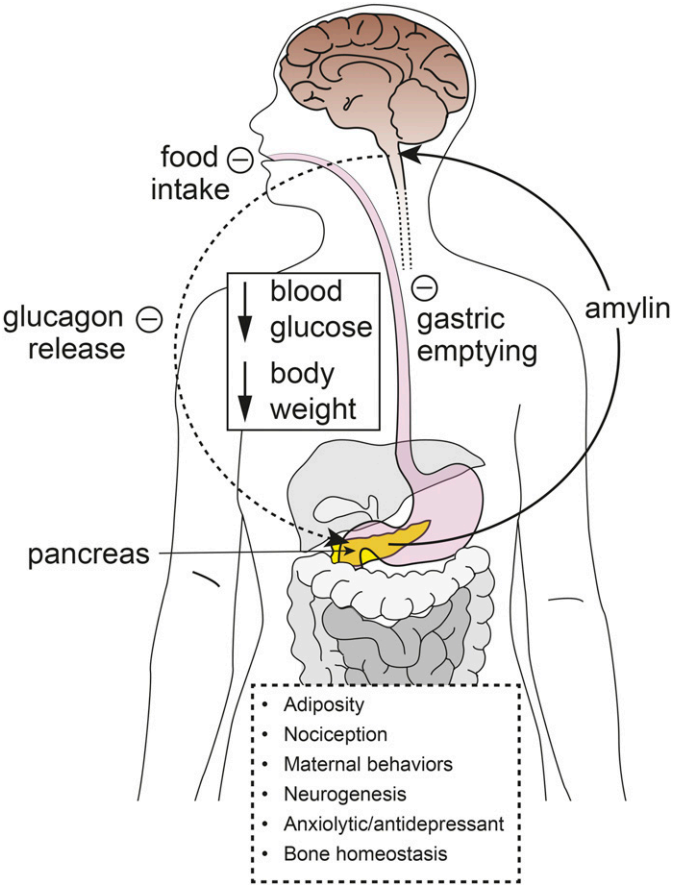

Fig. 2. An overview of the major actions of amylin. Amylin, secreted from the pancreas after a meal, circulates in the blood to activate specific receptors in the brainstem. This results in suppression of glucagon release from the pancreas, a reduction in food intake, and gastric emptying. The net effect of these actions is to decrease blood glucose, associated with longer-term reductions in body weight. The most well established effects of amylin are shown in the box with a solid line. Less well defined effects of amylin are shown in the dashed box.

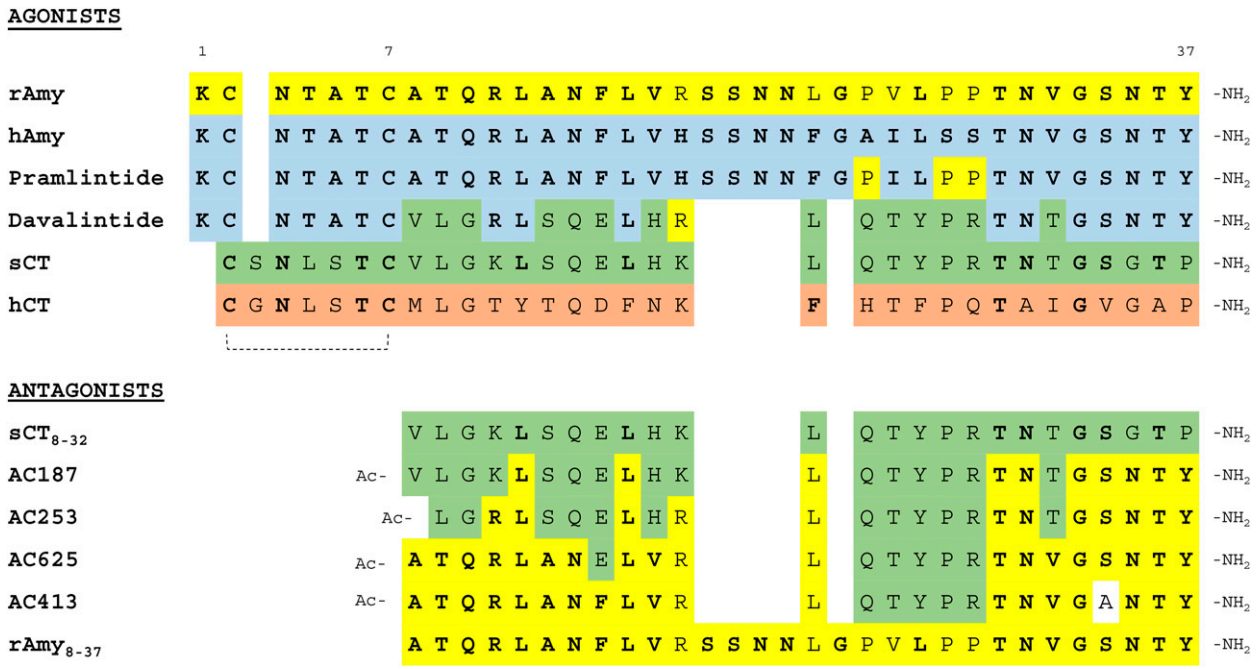

Fig. 3. Amino acid sequences of amylin and related peptides. The disulfide bond between N-terminal cysteine residues is indicated as a dashed line. All peptides have a C-terminal amide (NH_2) and some are acetylated at the N terminus (Ac). Human amylin (hAmy) is colored blue and conserved residues between hAmy and other peptides are in bold. Rat amylin (rAmy) is yellow, salmon calcitonin (sCT) is green, and human calcitonin (hCT) is orange. Many peptides are chimeras, as indicated by the coloring.

amidated C terminus. This is also the case for calcitonin, adrenomedullin, and adrenomedullin 2. Together, these peptides form a small family, united by these characteristic features. Consequently, there is a degree of overlap in binding the cognate receptors for each peptide and pharmacological activity.

Commonalities and differences in amino acid sequence and pharmacology between these peptides and across species have been used to develop agonists or antagonists that mimic or block amylin activity. The most notable of these is pramlintide, which is a relatively stable, bioactive synthetic analog of human amylin. Pramlintide is at least as potent as human amylin and is an amylinomimetic agent. It is also a 37-amino-acid polypeptide and differs in amino acid sequence from human amylin by replacement of amino acids with proline at positions 25 (alanine), 28 (serine), and 29 (serine) (Fig. 3). As a result of these substitutions, pramlintide is soluble, nonadhesive, and nonaggregating, thereby overcoming a number of the

TABLE 1
Summary of clinically relevant or commonly used amylin-related peptides and their effects

Sequences are shown in Fig. 3.

Peptide	Alternative Names	Agonist/ Antagonist	Pharmacology
Pramlintide	Symlin, tripro-amylin (human), $^{[Pro25,28,29]}$amylin (human), AC137	Agonist	Modified human amylin that lacks amyloidogenic properties; approved in the United States for clinical treatment of type 1 and type 2 diabetes; exhibits similar pharmacology and pharmacokinetic properties to nonamyloidogenic rat amylin. Pharmacology at molecularly defined amylin receptors shows close similarity to human and rat amylin (Gingell et al., 2014)
Davalintide	AC2307	Agonist	Longer-acting amylin agonist optimized for therapeutic treatment of obesity (Mack et al., 2010). Binds with high affinity to rat nucleus accumbens amylin and calcitonin receptors. Pharmacology at molecularly defined amylin receptors has not been reported
AC187		Antagonist	Used as a pharmacological tool to block endogenous actions of amylin. Receptor pharmacology studies show that this peptide can antagonize both calcitonin and amylin receptors (Hay et al., 2005)
AC253	$^{[Arg4,11]}$AC187	Antagonist	Similar to AC187, AC253 has been used to block endogenous actions of amylin (Mather et al., 2002). Receptor pharmacology studies show that this peptide can antagonize rat nucleus accumbens amylin and calcitonin receptors. Pharmacology at molecularly defined amylin receptors has not been reported
Salmon calcitonin 8-32	AC66	Antagonist	Used as a pharmacological tool to block endogenous actions of amylin. Receptor pharmacology studies show that this peptide can antagonize both calcitonin and amylin receptors (Hay et al., 2005)

physicochemical liabilities of native human amylin (Janes et al., 1996; Young et al., 1996b). Pramlintide exhibits similar biologic activity to that of amylin. Additional peptides are described in section III.B as well as in Fig. 3 and Table 1.

B. Amylin Expression, Secretion, and Metabolism

The β-cells found within the islets of Langerhans of the pancreas are the most well known site for amylin production. The discovery of amylin was made here, in the form of amyloid fibrils observed in patients with type 2 diabetes and in diabetic cats (Westermark et al., 1986, 1987a,b; Cooper et al., 1987). The pathophysiological role of amylin-containing fibrils has received much attention and was recently reviewed (Westermark et al., 2011; Abedini and Schmidt, 2013). Therefore, these fibrils will not be considered further in this review. It is the activity of amylin as a physiologically important circulating hormone that warrants this review.

The isolated-perfused pancreas, primary islets, or β-cell models have shown that amylin secretion can be stimulated by many factors, including glucose, arginine, and fatty acids, and that amylin secretion tracks insulin secretion (Kanatsuka et al., 1989; Ogawa et al., 1990; Qi et al., 2010). Pancreatic β cells are the major source of circulating plasma amylin, which ranges from 3–5 pM in the fasting state to postprandial concentrations of 15–25 pM (Lutz, 2006; Boyle et al., 2011). Consequently, amylin is cosecreted with insulin in a consistent molar ratio of approximately 15:1 (insulin: amylin). In a study of healthy women, an oral glucose load produced a significant rise in plasma amylin within 15 minutes. Fasting decreased plasma amylin concentrations (Hwang et al., 2008). Consistent with the concept of an elegant and multifactorial hormonal control system that controls metabolism, the incretin glucagon-like peptide 1 (GLP-1) can increase plasma amylin concentrations in healthy subjects (Asmar et al., 2010). Meal-associated fluctuations of circulating amylin levels are thought to directly reflect changes in β-cell secretion; the contribution of other amylin secreting cells to circulating amylin levels is considered minor. Furthermore, fluctuations in β-cell secretion, like the increases observed during the postprandial period, are believed to elicit the physiologic effects of amylin on eating and energy homeostasis (Lutz, 2010). Changes in circulating concentrations of amylin in obesity are covered later in this review. It is unclear whether amylin synthesis and secretion (either at baseline or postprandially) are altered by age because this aspect has not been studied systematically. The same is true for amylin's effects across different age ranges, but amylin's effect on eating has been shown to be present in young and old rats (Lutz et al., 1994).

Additional sites of amylin gene expression are neuroendocrine cells of the mammalian stomach (including humans), the dorsal root ganglion (Gebre-Medhin et al., 1998b; Tingstedt et al., 1999; Zaki et al., 2002), and the developing kidney (Wookey et al., 1998). Detailed information about the function of amylin derived from these sources is not available. However, given the important effects of amylin on food intake and gastric emptying, the intriguing observation that amylin binding sites (see sections III and V) are also present in the stomach warrants further investigation.

Amylin and pramlintide both have a circulating plasma half-life of approximately 13 minutes in rats after an intravenous bolus injection (Young, 2005d). Pramlintide pharmacokinetics is discussed in more detail in section X.A. As with many secreted peptides, amylin (and pramlintide) is metabolized primarily via the kidneys, most likely by proteolytic degradation, and nephrectomized rats exhibit a marked decrease in plasma clearance of amylin (Leckström et al., 1997; Vine et al., 1998a). In vivo, both amylin and pramlintide are rapidly cleaved to their respective des-Lys metabolites. These N-terminally truncated metabolites are both fully active as amylin agonists, and they exhibit similar in vivo potency and efficacy to the full-length peptides (Center for Drug Evaluation and Research Approval Package for Application Number 21-332. Clinical Pharmacology and Biopharmaceutics Review. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/21-332_Symlin%20Injection_biopharmr.pdf). Other amylin cleavage products are found in plasma, including amylin$_{17–37}$, which is likely to be inactive (Nakazato et al., 1990). It is not clear whether a strategy to inhibit mechanisms of amylin metabolism would enhance plasma amylin levels sufficiently as an alternative clinical intervention to exogenous amylin agonist therapy. Approaches to enhancing amylin agonist circulating half-life are covered in section VIII.D.

III. Amylin Receptors

A. Molecular Composition

Amylin receptors belong to the large superfamily of cell surface G protein–coupled receptors (GPCRs) (Poyner et al., 2002). This family is further subdivided; amylin receptors reside in class B. A prominent feature of amylin receptors is their close relationship to calcitonin receptors. This is an important consideration when interpreting the pharmacology, expression, and physiologic relevance of amylin receptors.

Some of the earliest attempts to characterize amylin receptors focused on the observations that amylin could displace CGRP binding and activate CGRP receptors in liver membranes or other preparations that endogenously expressed CGRP receptors. Cross-reactivity of amylin with CGRP receptors was plausible because these two peptides share approximately 40% amino acid sequence identity. Much of this early work has now been disregarded because the concentrations of amylin needed to displace CGRP binding

were often too high for this receptor to be physiologically relevant to amylin action. However, it may be important to reconsider some of this work in light of the now-known molecular identity of amylin receptors (see section III.B). For instance, a pivotal 1988 study describing salmon calcitonin–sensitive CGRP receptors, which we now know were probably amylin receptors, is an important example of the value of these earlier studies in understanding amylin receptor biology (Sexton et al., 1988).

To identify additional candidates for amylin receptors, radioligand binding assays have been developed to determine the quantity of specific amylin binding in different tissues (Bhogal et al., 1992; Beaumont et al., 1993). High levels of amylin binding are present in the lung, stomach fundus, spleen, and brain. To date, a similar study has not been conducted in human tissues.

Further investigation of amylin binding sites in the brain began to show overlap with calcitonin binding sites, and dense binding sites are detected in the nucleus accumbens, the area postrema (AP) of the brainstem, and the hypothalamus. Subsequent work showed that amylin could stimulate cAMP production in cells expressing calcitonin receptors and both amylin and calcitonin could be cross-linked to calcitonin receptors (Perry et al., 1997). A crucial observation was made that amylin did not interact with all cells that expressed calcitonin receptors and other factors were probably required for this to occur.

In 1999, two groups reported that the single transmembrane spanning receptor activity–modifying proteins (RAMPs) were the answer to this conundrum (Christopoulos et al., 1999; Muff et al., 1999). Although the calcitonin receptor does have some affinity for and can be activated by amylin, the presence of RAMPs can enhance the binding and potency of amylin to such a degree that postprandial concentrations of amylin that are in the picomolar range can produce functional responses (Tilakaratne et al., 2000). There are three RAMPs and several splice variants of the calcitonin receptor; consequently, there are many possible amylin receptor subtypes with potentially unique pharmacology, signaling, and regulation profiles. Another important consideration is that there are species differences in terms of pharmacology and receptor splice variants (Poyner et al., 2002). There has been no investigation as to the potential role of amylin receptors in the clearance and degradation of amylin, and as described in section III.D, it is not even clear whether amylin receptors are internalized. Furthermore, whether amylin degradation at the cellular level is also important as a mechanism to terminate receptor signaling is not known.

B. Pharmacology

Amylin receptors are heterodimers of the calcitonin receptor and a RAMP (Fig. 4; Table 2). The calcitonin receptor represents the core receptor protein that obtains high-affinity amylin binding properties by coexpression of one of the three RAMPs in the same cell (McLatchie et al., 1998; Christopoulos et al., 1999; Muff et al., 1999). RAMPs alter calcitonin receptor pharmacology from calcitonin-preferring to amylin-preferring receptors. The pharmacology of these receptors is summarized in Table 2. RAMPs can regulate the transport to the cell surface of other receptors with which they interact, but this is not reported to occur with the calcitonin receptor (Christopoulos et al., 2003; Hay et al., 2005). The precise site(s) of interaction between the calcitonin receptor and RAMPs is not yet known.

Numerous splice variants of the calcitonin receptor have been reported, which differ between species (Sexton et al., 1999). Only a few of these have been characterized with respect to amylin receptor pharmacology; however, on the basis of these observations, it is likely that their coexpression with RAMPs could also yield amylin receptors. The most common splice variant, and the one upon which most research has focused, is now known as $CT_{(a)}$ (formerly CTR2, CTR_{I1-}), as ratified by the relevant Nomenclature Committee of the International Union of Pharmacology (Poyner et al., 2002). This is the insert negative form of the receptor that has equivalent receptors in humans

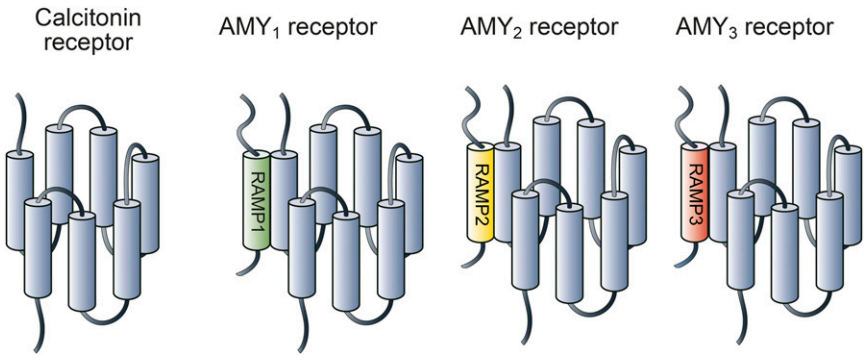

Fig. 4. A schematic diagram of the amylin receptors, which are formed by the interaction of the calcitonin receptor with RAMP1, RAMP2, or RAMP3 to generate the AMY_1, AMY_2, or AMY_3 receptors.

TABLE 2
Summary of the pharmacology of calcitonin and amylin receptors

Data are based on Alexander et al. (2013) and Gingell et al. (2014). Note that amylin receptor pharmacology can be influenced by calcitonin receptor splice variant and cell background (e.g., G protein expression level, expression of other RAMPs).

Receptor	Subunits	Agonist Potency Order
CTR	$CT_{(a)}$	sCT ≥ hCT ≥ rAmy,hAmy,pramlintide,CGRP
$AMY_{1(a)}$	$CT_{(a)}$ + RAMP1	sCT ≥ rAmy,hAmy,pramlintide ≥ CGRP > hCT
$AMY_{2(a)}$	$CT_{(a)}$ + RAMP2	Cell-type dependent
$AMY_{3(a)}$	$CT_{(a)}$ + RAMP3	sCT ≥ rAmy,hAmy,pramlintide > CGRP ≥ hCT

CTR, calcitonin receptor; h, human; r, rat; s, salmon.

and rodents. The pharmacology of calcitonin and amylin receptors is regularly updated as new literature becomes available via the joint initiative of the Nomenclature Committee of the International Union of Pharmacology and the *British Journal of Pharmacology Guide to Receptors and Channels* (http://www.guidetopharmacology.org).

A complication with the interpretation of many pharmacological studies of these receptors is that most of this work has likely used a mixed population of receptors, encompassing RAMP-coupled calcitonin receptors as well as calcitonin receptors alone. This means that although in binding assays human calcitonin has low affinity for [^{125}I]amylin binding sites, cells transfected with calcitonin receptors and RAMPs display potent human calcitonin functional responses. In some instances, the expression of RAMP3 with the calcitonin receptor results in a reduction in human calcitonin potency (Armour et al., 1999; Hay et al., 2005). The explanation for this is not clear but it could involve a modification to the conformation of the calcitonin receptor or a reduction in the number of "free" calcitonin receptors (Armour et al., 1999; Hay et al., 2005). Salmon calcitonin, on the other hand, has high affinity for [^{125}I]amylin binding sites.

The prevailing consensus is that the AMY_1 receptor is formed by calcitonin receptors expressed with RAMP1, generating a receptor with high affinity for amylin, salmon calcitonin, and CGRP (Poyner et al., 2002). In the case of the delta 47-splice variant of the calcitonin receptor, its expression with RAMPs results in a receptor that is activated with greater potency by CGRP than amylin (Qi et al., 2013). The formation of an AMY_2 (calcitonin receptor/RAMP2) receptor is highly dependent on cell background and calcitonin receptor splice variant (see section III.C on signaling). The AMY_3 receptor (calcitonin receptor/RAMP3) has high affinity for amylin and salmon calcitonin and lower affinity for CGRP. Although most studies have been conducted with rat amylin, a recent report shows that human amylin and pramlintide have very similar pharmacology to rat amylin at each receptor complex (Gingell et al., 2014).

The activity of CGRP at receptors that are classified as amylin receptors is intriguing and may underlie some of the difficulties in interpreting early studies of both CGRP and amylin pharmacology. For example, it has been suggested that the AMY_1 receptor could be a molecular explanation for reports of a "$CGRP_2$" receptor (Hay et al., 2008). A key question is what the physiologic relevance of each amylin receptor subtype is, and in particular what role the AMY_1 receptor might play in amylin and/or CGRP biology. Furthermore, salmon calcitonin is often used as an amylin agonist, but it is also a calcitonin receptor agonist; therefore, in in vivo studies, its effects may be mediated via both amylin and calcitonin receptors. Therefore, reference to salmon calcitonin derivatives as "amylinomimetics" should be treated with caution because it is not known that all activities are occurring directly through amylin receptors.

Antagonists are important tools for determining the contribution of amylin to a particular physiologic function or for determining the receptor via which amylin acts. Several peptides have been reported as amylin receptor antagonists, including salmon calcitonin 8-32 and AC187 (Fig. 3), and they are widely used in the literature (Table 1). Characterization of these antagonists at molecularly defined calcitonin and amylin receptor subtypes shows that they are able to antagonize both receptor classes and, therefore, should be used carefully. However, when used at appropriate concentrations to block the endogenous actions of amylin, their effects are likely to be specific to amylin receptors. However, these antagonists do not have sufficient subtype selectivity to be able to determine the specific amylin receptor that may be mediating a given effect (Hay et al., 2005; Bailey et al., 2012).

The mechanism of amylin binding to its receptors is likely to follow the general scheme of class B GPCRs in which the peptide C terminus engages with the receptor extracellular domain, whereas the disulfide ring at the N terminus triggers receptor activation through engagement with the transmembrane portion of the receptor (Archbold et al., 2011). However, little is known about the details of this for amylin specifically and what the relative role of calcitonin receptor or RAMP is in peptide engagement. Based on recent crystal structures of the CGRP and adrenomedullin receptors, it is anticipated that amylin will bind in the most part to the calcitonin receptor, with a small contribution from RAMP (Booe et al., 2015). Studies using RAMP1/RAMP2

chimeras and site-directed mutagenesis have clearly identified that the N terminus of RAMP is important for peptide interactions (Udawela et al., 2006a,b; Qi et al., 2008). A specific RAMP1 residue (tryptophan 84) may directly contribute to amylin and CGRP interactions with the AMY_1 receptor (Gingell et al., 2010; Booe et al., 2015). Some data for CGRP at the AMY_1 receptor show that a conserved threonine at position 6 in the peptide is essential for receptor activation (Hay et al., 2014). An alanine analog of amylin at this position had abolished efficacy in vivo, implying a similar effect at amylin receptors (Roth et al., 2008b).

C. Signaling

Most studies of amylin receptor signaling derive from transfected model cellular studies. These studies show that RAMPs can influence the ability of the calcitonin receptor to interact with different G proteins (Morfis et al., 2008). In terms of molecular mechanisms of amylin receptor signaling, the RAMP C terminus is important for defining G protein coupling (Udawela et al., 2006a,b, 2008). The calcitonin receptor splice variants also have different capacities to interact with intracellular signaling molecules (Poyner et al., 2002). The resulting complexity in amylin receptor signaling is substantial.

Consistent with amylin receptors containing the core GPCR, the calcitonin receptor, amylin results in acute activation of Gs-coupled pathways, resulting in an acute increase in intracellular cAMP. Gq coupling has also been reported and acute phosphorylation of extracellular signal–regulated kinase 1 and 2 (ERK1/2) is also evident, which may relate to protein kinase C rather than protein kinase A activation (Morfis et al., 2008).

However, many of these experiments are difficult to interpret because of the intrinsic ability of amylin to activate the calcitonin receptor. Therefore, mixed populations of free and RAMP-complexed calcitonin receptors, which are likely to exist in many experimental systems, may be activated simultaneously by amylin (depending on the concentration applied). Thus, the precise receptor complex through which amylin results in activation of a particular signaling pathway is often unknown. For phosphorylation of ERK1/2 and increases in intracellular Ca^{2+}, amylin can certainly trigger these events but interestingly, the potency of amylin only increases slightly in the presence of RAMP when measuring these pathways compared with cAMP (Morfis et al., 2008; Qi et al., 2013). Nevertheless, the time course of rapid ERK1/2 phosphorylation by amylin in cells transfected with calcitonin and amylin receptors is consistent with its time course for activation in AP neurons (see section VI.B) and osteoclasts (Dacquin et al., 2004; Morfis et al., 2008; Potes et al., 2012; Qi et al., 2013). Activation of ERK1/2 is clearly a pathway that amylin can use, but more work is needed to determine the precise circumstances under which this occurs and the mechanism involved. There has been no specific investigation of whether amylin-induced ERK activation is G protein dependent or arrestin mediated; however, the calcitonin receptor can recruit arrestin in response to human and salmon calcitonin (Andreassen et al., 2014). Amylin was recently reported to either increase or decrease ERK and Akt phosphorylation in dispersed islets, depending on the amylin and glucose concentration (Visa et al., 2015).

cGMP has also been linked to amylin's effects in the AP but has not been studied at defined amylin receptor subtypes in cell lines (Riediger et al., 2001). Conversely, cAMP has not been measured in response to amylin in AP neurons but is the prototypical pathway measured in transfected cell studies. Therefore, there is still a great deal of research needed to clarify which intracellular signals are triggered by amylin within its target cells, and which of these signals are necessary or sufficient to mediate amylin's effects.

D. Regulation

GPCR signaling is carefully regulated to maintain cellular responsiveness to natural ligands. Acutely, this can be in the form of receptor phosphorylation, uncoupling from signaling proteins, and removal of the receptor from the cell surface (internalization), followed by recycling of the receptor back to the cell surface or degradation of the receptor. In the longer term, such as in the case of prolonged exposure of the receptor to an agonist drug, the synthesis of the receptor protein can be downregulated (Luttrell, 2006). The short- or long-term regulation of defined amylin receptor subtypes has not been studied. However, there is evidence that the calcitonin receptor can be regulated by these mechanisms in a cell- and ligand-dependent manner. The calcitonin receptor is also subject to downregulation, influencing the utility of calcitonin agonists as drugs for bone diseases (Tashjian et al., 1978; Bouizar et al., 1987). Although it is tempting to infer that amylin receptors will behave similarly, these processes are highly dependent on cellular context; thus, how the calcitonin receptor behaves in osteoclasts is unlikely to translate directly to other cell types, such as AP neurons. Importantly, although this has not been studied with the calcitonin receptor, RAMPs are known to alter receptor fate in terms of regulation; therefore, the amylin receptors could be regulated quite differently from the calcitonin receptor alone (Bomberger et al., 2005a,b). Continuous infusion studies of 3–7 days with osmotic minipumps in rats and mice show that although there is a gradual decline in effect on food intake over time, amylin responsiveness is still evident, suggesting that receptor responsiveness is maintained under these conditions and that fluctuations of circulating amylin levels will still trigger relevant effects (Isaksson et al., 2005; Roth et al., 2006; Mack et al., 2007; Boyle et al.,

2011; Olsson et al., 2012). This is also apparent during conditions of prolonged hyperamylinemia (associated with hyperinsulinemia), such as type 2 diabetes and/or obesity, in which administration of exogenous amylin analogs remains effective to produce both glucoregulatory and weight-lowering actions (Boyle et al., 2011). There is little evidence that responsiveness to pramlintide wanes over time in clinical studies. In fact, pramlintide (two to three daily injections) consistently produces significant and durable actions to decrease body weight in obese patients for up to 12 months (Smith et al., 2008). Although these changes do plateau in the mid-single-digit percentage range, whether this reflects direct receptor desensitization and/or the emergence of counter-regulatory mechanisms to a weight/calorie-reduced state is difficult to dissect.

E. Expression and Physiologic Relevance of Amylin Receptor Subtypes

Although there are many potential amylin receptor subtypes, discerning which subtype is most relevant to each action of amylin is a considerable challenge. A range of factors, including a lack of receptor-selective pharmacological tools, the heteromeric nature of the receptors, their discrete neuronal expression, and a lack of reliable or widely available RAMP antibodies all contribute to this absence of information. It is also important to point out that RAMPs have several other GPCR partners, so their expression is only indicative of a potential interaction with the calcitonin receptor (McLatchie et al., 1998; Christopoulos et al., 2003; Harikumar et al., 2009; Lenhart et al., 2013).

Amylin's actions are mediated by a membrane-bound receptor that is present in the AP, the presumed primary site for most amylin actions (see section VI). Amylin binds strongly to the AP, and calcitonin receptor mRNA and protein are reported to be expressed here (Beaumont et al., 1993; Sexton et al., 1994b; Nakamoto et al., 2000; Barth et al., 2004; Becskei et al., 2004). RAMP1, RAMP2, and RAMP3 are all stated to be present in the AP, but thus far only data from in situ hybridization studies are available (Ueda et al., 2001; Stachniak and Krukoff, 2003). Amylin-induced neuronal activation, as assessed by c-Fos mRNA, colocalizes with $CT_{(a)}$ and RAMP3 mRNA in the rat AP (Sexton et al., 1994b; Christopoulos et al., 1995; Barth et al., 2004; Becskei et al., 2004). Laser capture microscopy will be useful for demonstrating that all necessary amylin receptor components are in fact expressed in single AP neurons or other types of neurons (Le Foll et al., 2015). This will also be a useful technique for determining which may be the most relevant amylin receptor subtype in different brain areas.

However, despite the evidence that all structural components of functional amylin receptors are present in the AP and that the AP is necessary for amylin action (see below), it still remains to be shown that the calcitonin receptor protein colocalizes with any specific RAMP in amylin-sensitive AP neurons. Furthermore, whether site-specific ablation of one of the critical components of the functional amylin receptor in AP neurons is sufficient to abolish amylin action has not been tested. Neural RAMP1 overexpression can augment amylin activity (see section V.C), suggesting that the AMY_1 receptor subtype could be particularly important. Calcitonin-sensitive CGRP binding sites in the AP and nucleus accumbens support this hypothesis (Sexton et al., 1988). However, the data are insufficient to make judgement calls about which receptor might be most effectively targeted with amylin agonists.

Other potential sites of relevance to amylin biology include the subfornical organ (SFO), nucleus of the solitary tract (NTS), ventral tegmental area (VTA), nucleus accumbens, and hypothalamus. Amylin binding has been reported in each of these brain areas, accompanied by calcitonin receptor protein and/or mRNA (Olgiati et al., 1983; Sexton et al., 1994a,b; Christopoulos et al., 1995; Hilton et al., 1995; Skofitsch et al., 1995; Nakamoto et al., 2000; Ueda et al., 2001; Stachniak and Krukoff, 2003; Barth et al., 2004; Becskei et al., 2004; Eftekhari and Edvinsson, 2011). The presence of RAMPs and thus functional amylin receptor subtypes is subject to the same caveats described above. Nevertheless, the SFO reportedly expresses mRNA for all three RAMPs, whereas the NTS expresses only low levels of RAMP2. Hypothalamus expression of RAMP1 and RAMP2 is evident in several nuclei. RAMP2 mRNA has been reported in the preoptic hypothalamic area, suprachiasmatic nucleus, paraventricular nucleus, periventricular nucleus, supraoptic nucleus, arcuate nucleus, ventromedial nucleus, and dorsomedial nucleus. RAMP1 has been reported in fewer hypothalamic nuclei, namely the arcuate nucleus, ventromedial nucleus, and dorsomedial nucleus (Oliver et al., 2001; Stachniak and Krukoff, 2003). Recently, all three RAMPs were detected in the ventromedial hypothalamus (VMH) (Le Foll et al., 2015). In the nucleus accumbens, only RAMP1 has been reported. The VTA was recently reported to express all necessary receptor components and is suggested to be another area of physiologic amylin relevance (Mietlicki-Baase et al., 2013). All of these are rodent studies. There is no information on the expression of RAMP2 or RAMP3 protein in the human brain and there are very limited data for RAMP1, as a consequence of it being a subunit of the CGRP receptor, which is a major target for treating migraine (Edvinsson and Warfvinge, 2013). There are some primate data for calcitonin receptor expression, and calcitonin receptor expression was recently shown in the human brainstem (Walker et al., 2015). This appeared to overlap with RAMP1 in several places; however, there was limited colocalization performed. Nevertheless, the coexpression of both proteins was found in some blood vessels and in the spinal trigeminal tract (Walker et al., 2015). This is the first

report of AMY_1 receptor expression at the protein level in human tissues. Overall, the current data are unsatisfactory due to the challenges outlined in the opening paragraph of this section, and specific amylin receptor subtypes cannot be attributed to amylin activities at the present time.

IV. Genetic Models

A number of transgenic and knockout models have been used to shed light on the role of amylin and its cognate receptors. Key findings from these models are summarized in Table 3 and each model is reviewed below.

A. Genetic Models of Amylin Deficiency or Overexpression

Amylin knockout animals have been developed to study the role of amylin in nutrient (in particular glucose) metabolism and bone metabolism (Gebre-Medhin et al., 1998a). These mice were established on a mixed 129Ola/B6 background in which the amylin coding sequence in exon 3 of the amylin gene was replaced by a neomycin resistance cassette. The specific phenotypes of these amylin-deficient mice that are not related to amylin's role in the control of eating include a lower bone mass due to a higher number of osteoclasts, and reduced late phase nociception in the paw formalin test; the latter implies that endogenous amylin increases the perception of chemical pain (Table 3), despite findings from pharmacology studies reporting that amylin agonists can have analgesic properties (Huang et al., 2010). Furthermore, these mice had increased insulin secretion and a more rapid glucose elimination, which may indicate that endogenous amylin exerts an inhibitory tone on insulin secretion (Gebre-Medhin et al., 1998a).

In the mixed genetic background, male amylin knockout mice show a slightly higher rate of body weight gain compared with corresponding wild-type controls (Gebre-Medhin et al., 1998a). This knockout model has also been backcrossed onto the C57/Bl6 background strain more commonly used in metabolic disease studies. As reported by Olsson et al. (2012), body weight did not differ between knockout and wild-type animals over a 27-week study. This study also tracked food intake, meal number, and meal size over this period and did not report any differences between groups. In another study, body weight, body composition, and plasma leptin levels were compared in male

TABLE 3
Summary of amylin-related transgenic and knockout models

Model	Genetic Background and Method	Reported Effects	Reference
Amylin gene deletion	129Ola/B6 (coding sequence of exon 3)	Lower bone mass/increased osteoclasts Some reduced nociception Increased insulin secretion/more rapid glucose elimination Transient weight gain Reduced sensitivity to anorexigenic effects of CCK	Gebre-Medhin et al., 1998a,b
	Above mice backcrossed onto C57/Bl6	Transient increase in adiposity in females Food intake, body weight unchanged (versus wild type) Reduced sensitivity to endogenous leptin Reduced hypothalamic leptin receptor mRNA	Turek et al., 2010
Amylin overexpression	FVB/n (pronuclear microinjection of construct into fertilized oocytes)	Diabetic Slight decrease in body weight (likely due to glycosuria, consequence of diabetes)	Wong et al., 2008
Calcitonin receptor deletion	Unclear. Deletion of exons 6 and 7 of *calcr*	High bone mass, increased bone formation (normal resorption)	Dacquin et al., 2004
	C57/Bl6. Cre-*Lox*P deletion of exons 13 and 14	Food intake and body weight not well evaluated	Davey et al., 2008
	C57/Bl6. Cre-*Lox*P deletion of exons 6 and 7	Increased bone formation	Keller et al., 2014
RAMP2 and RAMP3 knockout models		RAMP2 deletion is lethal; haploinsufficiency results in defects in bone homeostasis RAMP3 knockout models have reduced body weight with normal food intake	Dackor et al., 2007; Kadmiel et al., 2011
Neuronal RAMP1 overexpression	Multiple lines evaluated	Decreased body weight, adiposity and endogenous leptin Increased energy expenditure and sympathetic tone Increased BAT, UCP1, and UCP3 Enhanced sensitivity to exogenous amylin	Zhang et al., 2011

and female wild-type and knockout mice maintained on either a low-fat or high-fat diet (Turek et al., 2010). With the exception of a modest increase in adiposity in female knockout mice maintained on the low-fat diet, there were no consequences of amylin gene deletion; these results imply that at least in the endogenous system, mice can compensate for an amylin deficit.

Interestingly, amylin knockout mice on the 129Ola/B6 background differed from control animals in their interaction with other factors controlling eating, indicating that endogenous amylin may have a facilitating role for other controls of eating, such as cholecystokinin (CCK) (Mollet et al., 2003a). Likewise, low-fat–fed amylin knockout mice on the C57/Bl6 background were found to be less responsive to exogenous administered leptin. Leptin-induced p-signal activator of transcription 3 (STAT3) signaling and leptin receptor mRNA expression were reduced in the ventromedial and arcuate hypothalamus of amylin knockout mice, and the weight-lowering effects of minipump-administered leptin were attenuated (Turek et al., 2010). Together, these studies suggest a modest role for endogenous amylin in the overall control of energy balance, most likely via an effect on eating in interaction with other satiating and adiposity signals (Lutz, 2010).

Numerous rodent models with overexpression of human amylin have been generated to investigate the role of islet amyloid deposition in the destruction of pancreatic β-cells and the pathophysiology of diabetes (Westermark et al., 2011). To our knowledge, however, no detailed studies are available on the effect of amylin overexpression on eating or body weight. Rats that were transgenic for the overexpression of human amylin gained weight comparably until 5 months of age but then had a slightly lower body weight than wild-type controls (Butler et al., 2004). The authors argued that lower body weight may have been a consequence of energy loss due to the development of glycosuria and diabetes in the transgenic rats, but no detailed data on food intake were provided. Hence, reduced eating, which in principle would be consistent with the effects of exogenous amylin on eating and body weight, cannot be excluded. The rather small difference between transgenic and control animals may have been due to receptor desensitization or a redundant system controlling body weight. Because the major difference in body weight between amylin knockout and wild-type mice occurred mainly within the first 3 to 4 months of life, the same may also hold true for animals overexpressing amylin. Another recent article with mice that were transgenic for the overexpression of human amylin did not report data on eating and body weight (Wong et al., 2008).

B. Genetic Models of Amylin Receptors

Given the heteromeric nature of endogenous amylin receptors, the phenotypes of both calcitonin receptor– and RAMP-deficient/overexpressing animals need to be considered. In neither case can the phenotype be considered specific to amylin; models of altered calcitonin receptor expression will likely exhibit phenotypes relevant to both calcitonin and amylin, whereas with RAMPs the phenotype could be relevant to many hormonal systems.

Several models of altered calcitonin receptor expression have been generated. Deletion of exons 6 and 7 of the calcitonin receptor gene *calcr* results in no viable offspring (Dacquin et al., 2004). However $calcr^{+/-}$ mice were viable with expression of the receptor reduced by 2-fold by quantitative polymerase chain reaction. The genetic background of these mice was not clear. The phenotype reported was that of high bone mass due to increased bone formation, with normal bone resorption (Dacquin et al., 2004). These mice were cross-bred with $amylin^{+/-}$ mice, but neither data on eating nor data on body weight were reported. Because of the nonidentical phenotypes of $calcr^{+/-}$ and amylin-deficient mice, it was concluded that amylin may act through a receptor other than the calcitonin receptor. However, studies of this type are often very difficult to interpret due to the complicated receptor system and because sites of calcitonin receptor expression during development are not identical to those in adulthood, as evident from human calcitonin receptor/*Lacz* transgenic mice (Jagger et al., 2000). Subsequent studies have developed mice with 94%–100% deletion of the calcitonin receptor, using the Cre-*Lox*P system, targeting exons 13 and 14 or exons 6 and 7 (Davey et al., 2008; Keller et al., 2014). There were no differences in body weight in male or female mice compared with wild-type mice, although the ages at which these measurements were taken were not specified. The first attempt at osteoclast-specific deletion of the calcitonin receptor resulted in no viable offspring, using floxed calcitonin receptor mice crossed with TRACP-Cre mice (Davey et al., 2008). Later, however, a cross with cathepsin K-Cre transgenic mice resulted in viable mice. These mice showed increased bone resorption (Turner et al., 2011). Keller et al. (2014) also developed mice with osteoclast-specific disruption of *calcr*, which showed increased bone formation. Although these models give intriguing insight into the physiologic role of the calcitonin receptor in bone biology, none of these models have been carefully evaluated with respect to food intake, body weight, or sensitivity to diet-induced obesity.

Knockout models lacking each RAMP have been generated and their phenotypes were recently summarized (Kadmiel et al., 2012). Most work has focused on understanding their role in adrenomedullin or CGRP physiology. RAMP1 and RAMP3 global deletion leads to viable offspring, whereas loss of RAMP2 is lethal and therefore heterozygous mice are used to study its function. In all cases, there are no reports in relation to glucose homeostasis. Haploinsufficiency of RAMP2 results in defects of bone homeostasis (Kadmiel et al.,

2011). Only for RAMP3 is a body weight phenotype reported, whereby there is an age-dependent lean phenotype at approximately 6 months of age, compared with wild-type mice. Food and water intake was apparently unaltered in these animals and the reason for the difference in body weight is not yet known (Dackor et al., 2007). Because of the lack of investigation into parameters relevant to amylin, no conclusions can be drawn as to the relative importance of each RAMP at this time.

A few RAMP overexpression models have also been generated. There is a global human RAMP1-overexpressing model and a neural-specific human RAMP1-overexpressing model (Zhang et al., 2007; Sabharwal et al., 2010). Characterization of the second of these does potentially provide insight into amylin physiology and we describe the relevant aspects in section V.C. There is also a model of RAMP2 overexpression in smooth muscle, but this is unlikely to have particular relevance to amylin (Tam et al., 2006). Overall, the extant literature on RAMP transgenics has included only a limited investigation of each model with respect to amylin physiology.

V. Physiology of Amylin's Effect on Eating

This section focuses on animal studies that have helped to define the physiologic relevance of amylin, especially with respect to its action on energy homeostasis. Data are summarized about the effects of amylin on different aspects of energy homeostasis (i.e., its effect on eating as a satiating hormone and adiposity signal and its effect on energy expenditure). Because some reviews have recently summarized major aspects of amylin physiology (Lutz, 2012a,b; Young, 2012), this section focuses mainly on more recent articles that extend the perspectives previously described.

Amylin is an important regulator of nutrient fluxes because it reduces energy intake, modulates nutrient utilization by inhibiting postprandial glucagon secretion, and increases energy disposal by preventing compensatory decreases of energy expenditure in weight-reduced individuals. The most investigated function of amylin is to reduce eating by inducing satiation (the promotion of meal-ending processes). Whether amylin also influences satiety (the control of the intermeal interval) has not been tested formally. Amylin's satiation effect is thought to be mainly mediated by stimulation of specific amylin receptors in the AP. Secondary brain sites to mediate amylin action include the NTS and the lateral parabrachial nucleus (LPB), which convey the neural signal to the lateral hypothalamic area, and other hypothalamic nuclei (Fig. 5). Amylin may also signal the degree of adiposity because plasma levels of amylin are increased in adiposity and because higher amylin concentrations in the brain result in reduced body weight gain and adiposity, whereas amylin receptor antagonists increase body adiposity. The central mechanisms involved in amylin's effect on energy expenditure are much less known. A series of recent experiments in animals and humans indicate that amylin agonism is a promising therapeutic option for obesity, especially in combination with other hormones and/or small molecule anorectic agents. The most extensive data set is available for the combination therapy of amylin and leptin. Ongoing research focuses on the mechanisms of these interactions.

A. Amylin as a Satiation Signal

One of the most extensively investigated functions of amylin focuses on its role as a signal of satiation (Lutz, 2010). It is believed that amylin is a physiologic regulator of meal size (Geary, 2005; Lutz and Geary, 2008). Eating leads to a rapid increase in circulating amylin levels and exogenous amylin reduces eating within minutes after application. The effect of amylin in reducing meal size is dose dependent and the effect is effectively and specifically blocked by amylin antagonism; on the other hand, amylin antagonists alone increase eating by a meal size effect (Mollet et al., 2004). Importantly, amylin and its analogs reduce meal size without producing signs of conditioned taste aversion or visceral illness (Lutz et al., 1995b; Morley et al., 1997; Mack et al., 2007, 2010). Overall, the data indicate that amylin acts as a physiologic short-term satiating hormone in rats.

Reduced drinking that has been observed in some studies after the administration of exogenous amylin seems to be secondary to the reduction in eating. No evidence is available that would support the opposite conclusion (i.e., that amylin reduces eating secondary to an effect on water intake) (Lutz et al., 1994; Arnelo et al., 1996; Morley et al., 1997; Asarian et al., 1998).

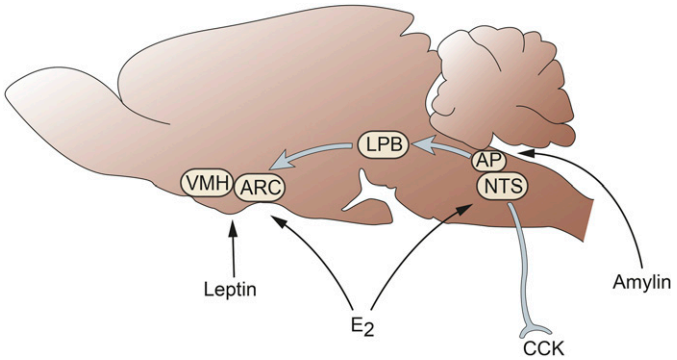

Fig. 5. An overview of the neuroaxis involved in mediating amylin's eating-inhibitory effect and presumed sites of interaction with other hormones, shown on a graphical representation of the rat brain. Peripheral amylin reaches the AP via the blood circulation and activates AP projection areas such as the NTS and the LPB. Amylin interacts with CCK, which signals the brain via vagal afferents, and with leptin. Estradiol modulates amylin's effect but it is currently unclear whether this may involve brainstem or hypothalamic structures. ARC, arcuate nucleus; E_2, estradiol.

B. Amylin as an Adiposity Signal

The concept of "homeostatic eating controls" classically distinguishes between adiposity or "tonic" signals that enhance the effect of satiation and other meal-associated or "episodic" signals; adiposity signals are secreted in proportion to body adiposity by adipose tissue (leptin) or the pancreas (insulin, amylin), and are thought to affect eating by enhancing the effects of satiation signals. For example, amylin, similar to leptin and insulin, enhances CCK's satiating effect (Riedy et al., 1995; Barrachina et al., 1997; Bhavsar et al., 1998; Mollet et al., 2003a). Even though the concept of distinct satiation versus adiposity signals is an oversimplification of the physiologic situation, it helps to structure the signals involved in the control of energy balance. The situation is, however, complicated by the fact that some signals actually may be part of both categories; insulin is considered a classic adiposity signal (Woods, 2005), but it is also released during meals, and blockade of endogenous insulin by insulin antibodies increased meal size (Surina-Baumgartner et al., 1995).

Like insulin, a similar "dual role" may also apply to amylin; it is released during meals and a number of experiments suggest a role for amylin as an adiposity signal. For example, basal plasma levels of amylin are higher in obese rats than in lean rats (Pieber et al., 1994), and high-fat–fed obese rats have higher baseline amylin levels than age-matched lean controls (Boyle et al., 2010, 2011). However, not all studies showed such an effect (Gloy et al., 2010) and it is possible that extended periods of being overweight may be required (Boyle et al., 2011) for baseline amylin levels to increase after the onset of obesity. In general, amylin secretion from the β-cell correlates very tightly with insulin secretion.

More direct evidence for amylin being an adiposity signal is provided by experiments showing that chronic peripheral (Roth et al., 2006; Mack et al., 2007) or central (Rushing et al., 2001) amylin decreases body weight and fat gain, whereas amylin antagonists increase body adiposity (Rushing et al., 2001). Furthermore, centrally administered amylin levels seem to directly influence the target body weight of rats (Wielinga et al., 2010). Together, these data suggest that central amylin, like leptin or insulin, may encode the regulated level of body weight and hence may contribute to the relative constancy of body weight throughout adult life.

Even though the underlying mechanisms are not yet clear, amylin action shows interesting differences to the action of CCK, which may be a "pure" satiation signal. First, rats receiving a continuous infusion of CCK do not show sustained reductions in eating or body weight (Crawley and Beinfeld, 1983). Second, CCK given before each spontaneous meal effectively reduced meal sizes, but the effect seems to be totally compensated by an increase in meal frequency (West et al., 1984). Similar compensations do not occur during chronic amylin infusions (Lutz et al., 1995b; Arnelo et al., 1996), perhaps because of the tonic eating-inhibitory effect of amylin (Grabler and Lutz, 2004). It would be interesting to know whether tonic and episodic effects of amylin rely on similar or distinct mechanisms. Both, however, seem to require an intact AP (Lutz et al., 1998c, 2001).

C. Amylin and Energy Expenditure

In some of the studies mentioned above, chronic amylin reduced body fat more than in pair-fed controls (Roth et al., 2006), as also shown in other experiments (Isaksson et al., 2005). This indicated that amylin infusions most likely reduced eating and increased energy expenditure (Mack et al., 2007; Wielinga et al., 2007, 2010). Hence, amylin shares another similarity with leptin because it influences energy balance by an eating and an energy expenditure effect (Isaksson et al., 2005; Roth et al., 2006; Mack et al., 2007; Osaka et al., 2008). In our own studies, amylin increased energy expenditure acutely when given at low doses into the third cerebral ventricle or directly into the AP (Wielinga et al., 2008, 2010). Furthermore, the decrease in energy expenditure that is the expected metabolic reaction in weight-reduced rats is prevented by chronic central amylin infusion (Wielinga et al., 2007, 2010).

Some questions in respect to amylin's effect on energy expenditure remain. First, it is not yet clear whether the effect is of physiologic relevance because the role of endogenous amylin in the control of energy expenditure has not been tested. Second, the site and neurotransmitter mechanisms of amylin's actions on energy expenditure also remain to be determined. Finally, further studies are necessary to define the underlying intracellular processes and whether these are similar to the ones activated in respect to amylin's effect on eating (e.g., pERK, cGMP).

Two recent studies provided interesting mechanistic input on amylin's effect on energy metabolism (Zhang et al., 2011; Fernandes-Santos et al., 2013). In rodents, as well as in humans, total energy expenditure depends on the thermogenic activity of the brown adipose tissue (BAT); BAT activity is controlled by the sympathetic nervous system and heat production relies on the organ specific expression of uncoupling protein (UCP)-1. Although earlier studies failed to demonstrate a clear effect of amylin on the expression of UCP-1 (Roth et al., 2006, 2008c), recent studies indicate that BAT activity is increased by amylin, that this effect is mediated by the sympathetic nervous system, and that the effects are enhanced in transgenic mice that overexpress RAMP1 of the AMY_1 receptor complex (Zhang et al., 2011; Fernandes-Santos et al., 2013). In brief, the neuronal overexpression of human RAMP1 led to sensitization of mice to the metabolic effects of

amylin; human RAMP1 transgenic mice were lighter, had less fat, had increased energy expenditure and body temperature, and showed at least temporary hypophagia compared with control animals (Zhang et al., 2011). The higher energy expenditure was mediated by an increased sympathetic tone in efferents subserving BAT, and this was associated with increased expression of the peroxisome proliferator–activated receptor γ coactivator 1a as well as UCP-1 and UCP-3 (Fernandes-Santos et al., 2013). Although these studies are presented in the context of amylin, the potential role of CGRP should also be considered in any study investigating RAMP1. This is because RAMP1 can form two high-affinity receptors for CGRP and CGRP knockout mice are reportedly protected against diet-induced obesity and have elevated core body temperature (Walker et al., 2010). It should be noted that CGRP can also decrease food intake (Lutz et al., 1998c; Dhillo et al., 2003).

VI. Mechanisms Underlying Amylin Actions

A. Amylin Action in the Brainstem

Several brain areas have high-affinity binding sites for amylin, as shown by amylin receptor autoradiography studies (Sexton et al., 1994b). Amylin binding is particularly strong in the circumventricular organs such as the SFO and the AP. Amylin action in the SFO may be linked to an effect of amylin to increase drinking (Riediger et al., 1999; Fry et al., 2007) but whether it is necessary for the expression of amylin's effect on eating has not been demonstrated.

In the context of amylin's effect on energy metabolism, the AP has received the most attention, although the contribution of other brain areas such as the VTA should also be considered (see below; Mietlicki-Baase et al., 2013). A transporter-mediated process may facilitate the transfer of amylin through the blood–brain barrier into the brain, also outside of the circumventricular organs (Banks et al., 1995; Banks and Kastin, 1998). Of note, the primary receptor sites for the eating-inhibitory effect of centrally administered amylin have thus far not been studied in great detail.

A large number of independent studies indicate that the AP plays an important role in mediating effects of peripheral amylin. Amylin appears to activate AP neurons by direct humoral action, whereas vagal or nonvagal afferents do not seem to be involved (Lutz et al., 1994, 1995a, 1998a,c, 2001; Morley et al., 1994; Mack et al., 2010). Furthermore, local amylin administration into the AP recapitulates peripheral amylin's effect, whereas the amylin antagonist AC187 has the opposite effect; AC187 also abolished the eating-inhibitory effect of peripheral amylin (Mollet et al., 2004). The AP also seems to be necessary for amylin's action to inhibit gastric emptying (Young et al., 1995; Gedulin et al., 1997; Wickbom et al., 2008; Mack et al., 2010; Young, 2005b). Electrophysiological and immunohistochemical studies supported a direct influence of amylin on the AP (Riediger et al., 2001, 2002, 2004; Potes et al., 2010b, 2012). As discussed in section III of this article, the receptor components (calcitonin receptor, RAMPs) necessary for amylin function appear to be expressed in the AP. The available evidence is not sufficient to specify which AMY receptor subtype is particularly important in the AP, or indeed in any part of the brain or other tissue.

B. Brainstem Mechanisms Mediating Amylin Signaling

A frequent marker to define stimulus-activated brain areas is based on the expression of the immediate early gene product c-Fos (Rowland et al., 1997; Becskei et al., 2004; Riediger et al., 2004; Mack et al., 2010; Potes et al., 2010a). It is important to note that c-Fos expression does not necessarily have a functional correlate for a behavioral effect of the same stimulus (e.g., see Züger et al., 2013). Nonetheless, c-Fos expression helps define brain areas that may be involved in responses to the respective stimulus. According to these studies, endogenous and exogenous amylin activates a neuroaxis (see section VI.D) comprising the AP, the NTS, and the LPB; activation of the latter areas seems to depend on an intact AP because the effect is absent in AP lesioned rats, and hence in rats that also do not eat less after amylin injection.

Markers that may also be functionally linked to amylin are the formation of the second messenger cGMP (Riediger et al., 2001) or the phosphorylation of ERK1/2 (Potes et al., 2012). Local AP injection of a membrane permeable analog of cGMP decreased eating similar to amylin by a meal size effect (Mollet et al., 2004). Furthermore, phosphorylation of ERK occurred rapidly in neurons carrying the calcitonin receptor after peripheral amylin (Lutz et al., 1995b; Potes et al., 2010b), and the prior administration of U0126 (1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene), an inhibitor of ERK phosphorylation, into the fourth brain ventricle reduced the acute eating inhibitor effect of amylin under certain experimental conditions (Potes et al., 2010b, 2012). Hence, cGMP and the ERK cascade seem to be directly involved in amylin's anorectic effect (see also section III.C).

A downstream consequence of amylin-induced activation of AP neurons seems to be an increased expression of dopamine-β-hydroxylase (DBH), the key enzyme in noradrenaline synthesis, and the subsequent release of noradrenaline possibly in the NTS or LPB (Potes et al., 2010c). DBH-positive neurons in the AP seem to be necessary for peripheral amylin to reduce eating because a partial chemical lesion of these neurons was sufficient to abolish the eating-inhibitory effect of peripheral amylin (Potes et al., 2010c). Interestingly, the number of amylin-activated neurons

correlated with the number of remaining noradrenergic neurons in the AP, indicating that rats with larger noradrenergic lesions and hence fewer remaining DBH-positive neurons also displayed lower numbers of amylin-activated AP neurons. A recent electrophysiological study suggested that glutamatergic neurotransmission in the AP seems to play a role in mediating amylin effects. According to this study, amylin receptors appeared to be located mainly on presynaptic glutamatergic terminals connecting to the AP neurons (Fukuda et al., 2013); the association of this effect with the effects in AP neurons described above has not yet been clarified.

C. Amylin-Sensitive Area Postrema Neurons Are Sensitive to Nutrients

The activity of amylin-sensitive AP neurons seems to be modulated by nutrients. The first evidence was provided by electrophysiological in vitro studies that showed that amylin and glucose coactivate AP neurons (Riediger et al., 2002); neurons that were activated by amylin reduced their spontaneous activity in a concentration-dependent manner when exposed to low glucose concentrations. This cosensitivity finds a correlate in an amylin-mediated effect that is reduced at low glucose levels; amylin's effect to reduce the rate of gastric emptying was absent under hypoglycemic conditions (Gedulin and Young, 1998). It will be interesting then to compare amylin's effects on eating under euglycemic, hyperglycemic, and hypoglycemic conditions. Teleologically, the glucose dependence of amylin's effects seems plausible because it guarantees that amylin should not limit the supply of nutrients at a time when they are urgently needed.

Amino acids have also been shown to modulate amylin's effect on eating and, at the cellular level on the activity of AP neurons. Interestingly, and opposite of the effect of glucose, amino acids seem to inhibit rather than activate these amylin responses. We recently showed that rats exposed to low-protein (1%–8%) diets reduce eating more when injected with amylin than rats exposed to a diet with normal protein content (18%). Similarly, dietary protein seemed to inhibit rather than to enhance the amylin-induced c-Fos activation in the AP, and the same was seen in rats that received parenteral injections of amino acids (Züger et al., 2013). The mechanisms underlying these phenomena and the functional implications are not clear; whether this mechanism is meant to prevent the intake of amino acid–deficient diets and thus amino acid imbalance is not clear and has not been tested because all rats included in these experiments were protein replete. In the same context, it is also not clear whether the effects are due to specific amino acids directly reducing the effect of amylin to activate AP neurons using AMY receptors themselves or via other cellular mechanisms. Finally, these studies also indicated that the effect of amylin to reduce eating and to induce c-Fos expression may be dissociated under certain conditions.

D. Neuroaxis Activation by Amylin

The evidence for an important (although perhaps not the sole [see below]) role of the AP for the mediation of the eating-inhibitory effect of peripheral amylin is strong (e.g., Sexton et al., 1994b; Lutz et al., 1998c; Becskei et al., 2004; Mollet et al., 2004; Mack et al., 2010; Potes et al., 2010c). The activation of AP neurons may then trigger the activation of a neuroaxis that projects rostrally to the forebrain and that includes the NTS, the LPB, and the central amygdala (CeA) (Lutz et al., 1998c; Becskei et al., 2007) (Fig. 5). Lesions of the respective brain areas (AP, NTS, and LPB) abolished amylin's effect, and activation (as indicated by c-Fos) was absent in brain areas rostral to the lesion (e.g., in the NTS, LPB, and CeA in AP lesioned rats, or in the CeA in LPB lesioned rats). The direct link between the respective brain areas was confirmed by the use of anterograde and retrograde neuronal tracers; these studies also identified the LPB as the primary relay between the hindbrain and the hypothalamus, including the lateral hypothalamic area, where amylin reduces fasting-induced c-Fos expression (Riediger et al., 2004; Potes et al., 2010a) and the VMH (Mollet et al., 2003b; Roth et al., 2008a; Turek et al., 2010). The latter area received more attention in recent years due to the possible role of the VMH in mediating the interaction between amylin and leptin (see section IX.A).

E. Amylin Action in Other Brain Areas

Despite the clear evidence indicating an important role of the AP in mediating peripheral amylin's actions, it also needs to be realized that amylin binding sites have widespread distribution throughout the brain (Sexton et al., 1994b), and the same is true for the expression of the amylin receptor components, the calcitonin receptor (Becskei et al., 2004) or the RAMPs (Ueda et al., 2001; Barth et al., 2004). Central amylin produces very potent effects on eating (e.g., Rushing et al., 2000; Osaka et al., 2008; Wielinga et al., 2010) that at least under certain conditions are independent of an intact AP (Lutz et al., 1998b), and most reports indicate that lower doses of amylin are required to reduce eating (and body weight) after infusion into the lateral or third than in the fourth cerebral ventricle (Lutz et al., 1997, 1998b; Rushing et al., 2000). Nonetheless, the physiologic relevance of the eating-inhibitory effect of central amylin is less clear, because for most of the potential sites of action, activation by peripheral amylin has not been demonstrated and it is also unclear which areas are primarily targeted by centrally infused amylin. Furthermore, evidence for an action of centrally produced amylin is scarce and may

only occur under very specific conditions in females, which may not be related to effects of amylin on eating (Dobolyi, 2009).

One interesting study recently provided evidence that amylin receptors in the VTA may be direct targets for peripheral amylin. The VTA is well known for its role in the control of eating and in particular for the intake of palatable food (Mietlicki-Baase et al., 2013). That study showed that all amylin receptor components are present in the VTA and that direct administration of the amylin/calcitonin receptor agonist salmon calcitonin into the VTA reduced eating of normal chow as well as palatable sucrose. Similar to peripheral amylin (Lutz et al., 1995b) or amylin given into the AP (Mollet et al., 2004), reduced eating appeared to result at least in part from a meal size effect. The authors suggested that amylin may reduce meal size by modulating the reward value of food throughout the meal. The observation that administration of the antagonist AC187 into the VTA increased eating and that intra-accumbens AC187 reduced the effect of peripheral salmon calcitonin on eating indicates that salmon calcitonin may in fact exert a direct action on VTA neurons and that the effects seem to be of physiologic relevance (Mietlicki-Baase et al., 2013). The relations between the VTA-mediated effect and the previously described AP-mediated effects are currently unclear. As noted in section III.C, salmon calcitonin and AC187 are not selective tools for only amylin receptors, having activity also at calcitonin receptors. Therefore, authors must be careful in their data interpretation using these molecules.

The nucleus accumbens, which also plays a role in reward/hedonics, has also been tested for its potential role in the control of food intake by amylin (Kelley, 1999; Baldo and Kelley, 2001). The experiments were based on the knowledge that the nucleus accumbens contains a high density of high-affinity amylin binding sites (Beaumont et al., 1993; Sexton et al., 1994b). In fact, nucleus accumbens membranes had originally been used to characterize the properties of amylin binding sites. It was shown that administration of amylin into the shell of the accumbens reduced eating and drinking; however, because amylin also markedly reduced locomotor activity and because the latter effects were observed at lower doses than the eating effect, these motor effects may perhaps be part of an amylin effect on the "satiety sequence," which includes a number of typical postprandial behaviors like rearing and cleaning. A direct contribution of the nucleus accumbens to the amylin effect on meal termination seemed to be less likely (Halford et al., 1998).

F. Summarizing Remarks

Overall, the neuroaxis that is activated by peripheral amylin or its analogs has been reasonably well defined (Potes and Lutz, 2010). Evidence for a critical role of specific hindbrain structures, in particular the AP, NTS, and LPB, is compelling; activation of these structures may then affect specific hypothalamic nuclei (Fig. 5), but their individual roles in the effects of amylin on eating are still not clear (Potes et al., 2010a; Trevaskis et al., 2010a; Turek et al., 2010). Furthermore, more studies are required to define the potential contribution of other circumventricular organs such as the SFO (Fry et al., 2007; Hoyda et al., 2009; Mimee et al., 2013). Recent studies indicate that in addition to the AP, peripheral amylin may activate a more complex and distributed network that includes the VTA (Mietlicki-Baase et al., 2013, 2015). However, how the VTA and the other brain areas may interact is also not clear. Furthermore, although central amylin produces potent eating-inhibitory effects, the brain structures mediating these effects and their relevance under physiologic conditions are not clear.

VII. Other Actions of Amylin and Potential Therapeutic Areas

The existence of amylin binding sites and components of amylin receptor complexes outside of the neuroaxis mentioned thus far as well as behavioral pharmacological studies implicate amylin in other activities. These suggest alternative potential therapeutic areas for intervention with amylin receptor modulators. Some of these findings were briefly described in earlier sections. Overall, the extant literature surrounding these areas is not as robust as for amylin's antidiabetic/obesity effects and is summarized in Table 4.

A. Amylin in the Cardiovascular System

In general, the majority of amylin's actions on the cardiovascular system are believed to be mediated by activation of CGRP receptors, widely expressed within the vasculature (for details, see Young, 2005a). These actions are believed to be largely pharmacological due to the high plasma concentrations required to observe a response. Consequently, in rodent models, bolus intravenous injection of amylin produces a potent vasodilatation to decrease arterial blood pressure, accompanied with short-term baroreflex increases in heart rate (Young et al., 1991). Little work has been reported investigating how well these actions translate to human pharmacology. However, it is likely that clinically relevant doses of pramlintide for treatment of diabetes or obesity are insufficient to increase plasma exposure to levels required to activate CGRP receptors in humans, because it is estimated that the EC_{50} for blood pressure lowering exceeds therapeutic pramlintide concentrations by three orders of magnitude. Confirming this hypothesis, 12-month dosing with pramlintide in humans did not significantly change blood pressure (Young et al., 1999).

TABLE 4
Reported effects of amylin that are not extensively covered in this review and their potential therapeutic area

Potential Therapeutic Area	Key Findings	References
Depression/anxiety	Reduced immobility in forced swim test Increased hippocampal neurogenesis Reduced restraint stress-induced sucrose consumption and hyperthermia Reduced marble burying	Roth et al., 2009; Turek et al., 2010
Memory enhancement	Increased retention under conditions of "weak" conditioning but impaired retention under "strong" conditioning in T-maze Improved learning and memory	Flood and Morley, 1992; Zhu et al., 2015
AD	Decreased brain Aβ levels Improved performance in memory and cognition in preclinical disease models Increased markers of synaptic formation and decreased markers of inflammation and oxidative stress within hippocampus	Adler et al., 2014; Zhu et al., 2015
Antipsychotic/schizophrenia	Intra-accumbens infusion reversed amphetamine-induced prepulse inhibition disruption	Baisley et al., 2014
Pain	Analgesic effects in models of visceral pain when administered peripherally Antinociceptive effects linked to reduced spinal c-Fos expression No effects on tail immersion when given centrally Amylin knockout mice have reduced nociception	Bouali et al., 1995; Gebre-Medhin et al., 1998b; Sibilia et al., 2000; Huang et al., 2010
Osteoporosis	In a streptozotocin (STZ) rat model of diabetic osteopenia, addition of amylin improved bone indices apparently by both inhibiting resorption and stimulating bone formation. Amylin knockout mice have increased bone resorption (decreased bone mass/density, trabecular bone volume) but normal osteoblast and bone formation rates Osteogenic actions depend on diabetic status (effective in low-dose STZ type 2 diabetic but not insulin-resistant preclinical models)	Cornish et al., 1998; Horcajada-Molteni et al., 2000, 2001; Dacquin et al., 2004; Gutierrez-Rojas et al., 2013

Direct cardiac inotropic effects, potentially CGRP receptor mediated, have been reported for amylin in isolated rodent cardiomyocytes (Bell and McDermott, 1995), isolated rat hearts (Kaygisiz et al., 2010), and isolated cardiac tissue from pigs (Saetrum Opgaard et al., 1999). However, because of the high concentrations required to observe efficacy, it is again unlikely that these actions are clinically relevant at therapeutic doses.

B. Amylin and Lipolysis

Given the observation that amylin administration decreases adiposity, the extent to which amylin exerts direct effects on BAT and white adipose tissue has been explored. In isolated adipocyte preparations, amylin stimulates neither lipolysis nor basal or insulin-stimulated rate of glucose incorporation into either CO_2 or triacylglycerol (Cooper et al., 1988; Lupien and Young, 1993; Roth et al., 2006). When epididymal fat pads were excised from amylin-treated animals, the basal rate of lipolysis did not differ relative to a vehicle-treated control group. Sustained in vivo administration of amylin also failed to augment the ex vivo lipolytic sensitivity of adipose tissue to adrenergic stimulation. Thus, at least in epididymal fat, amylin did not regulate basal or stimulated lipolysis. However, more recent in vitro studies reported that amylin activated multiple intracellular pathways in human adipose tissue such as the STAT3, AMP-activated protein kinase, Akt, and ERK signaling pathways (Moon et al., 2011). Of note, these effects were obtained at very high pharmacological levels of amylin in the culture media and the physiologic significance of these findings remains to be determined.

C. Amylin and Maternal Behaviors

Microarray studies detected a marked increase (approximately 25-fold) in amylin expression within the preoptic area of the hypothalamus of rat dams (Dobolyi, 2009). These mRNA findings were confirmed by quantitative polymerase chain reaction and in situ hybridization methods. Next, a series of experiments detailed the time course (during late pregnancy and throughout lactation) and potential mechanisms for amylin expression (Szabó et al., 2012). First, mRNA levels remained elevated as long as the pups were not removed from the dams. Amylin expression was also induced in maternally behaving (sensitized) nonlactating females but not in nonsensitized nulliparous females or in females that did not become maternal despite the sensitization procedure. This phenomenon was sex steroid hormone independent because ovariectomy had no effects. In other studies, mothers were also separated from their pups for 22 hours. On return of the pups, neuronal activation was found in the mother's preoptic area, with a distribution pattern similar to amylin-expressing neurons. The authors hypothesize that amylin may play a role in the physiologic regulation of maternal adaptations potentially endocrine and emotive, although these remain to be formally demonstrated.

D. Amylin and Stress-Induced Eating

Preclinical findings suggest that amylin agonism may be beneficial in the treatment of neuropsychiatric disease (see Table 4). Amylin exerted anxiolytic and antidepressive effects in several preclinical assays (reviewed in Roth et al., 2009). In a chronic stress model, the effects of amylin administration were assessed in rats that were given access to standard laboratory chow and sucrose solutions and were also exposed to daily restraint stress. Vehicle-treated rats increased their consumption of sucrose and increased visceral adiposity. By contrast, amylin-treated rats decreased their stress-induced sucrose consumption after restraint stress, while maintaining their intake of standard laboratory chow (Roth et al., 2009). Beneficial effects have also been noted in other preclinical stress models. For example, during recovery from social stress in a visible burrow system (VBS), during which a dominance hierarchy is formed among the males, rats display hyperphagia and gain weight preferentially as visceral adipose tissue. By proportionally increasing visceral adiposity, social stress may contribute to the establishment of metabolic disorder. Amylin was administered to rats fed ad libitum during recovery from VBS stress in an attempt to prevent hyperphagia and the resultant gain in body weight and fat mass. Amylin treatment reduced food intake, weight gain, and accumulation of fat mass in male burrow rats but not in male controls that spent time housed with a single female rather than in the VBS (Smeltzer et al., 2012).

E. Antipsychotic-Like Actions of Amylin

Recently, antipsychotic-like actions of amylin were demonstrated after central injections (Baisley et al., 2014). Amylin infusion into the accumbens shell, but not the dorsal striatum, reversed amphetamine-induced prepulse inhibition without affecting baseline startle. Prepulse inhibition is a commonly used cognitive test paradigm of the reaction of an organism, such as a mouse, to "startling" stimuli of different magnitude, such as a sound (Castagné et al., 2009). Coinfusion of AC187 blocked the ability of amylin to normalize amphetamine-induced prepulse inhibition disruption. These findings suggest that amylin receptors may be a potential target for the development of putative antipsychotics and future work should explore the potential utility of systemically administered amylin in this regard.

F. Amylin and Alzheimer's Disease

Two recent reports suggest that peripheral treatment with either amylin or pramlintide can have therapeutic effects in Alzheimer's disease (AD) (Adler et al., 2014; Zhu et al., 2015). First, lower amylin levels were noted among subjects with AD and mild cognitive impairment compared with the cognitively intact subjects (Adler et al., 2014). This led the authors to test whether chronic infusions of the amylin analog pramlintide would be beneficial in rodent models of AD. Chronic infusions of pramlintide in the senescence-accelerated prone mouse (a model of sporadic AD) improved performance in the novel object recognition task (assay for memory and cognition). These behavioral effects were accompanied by increased markers of synaptic formation and decreased markers of inflammation and oxidative stress within the hippocampus (Adler et al., 2014). Likewise, both amylin and pramlintide administration in a transgenic model of AD improved learning and memory in the Y-maze and Morris swim tests. These effects were coupled with reduced amyloid burden and reduced Aβ in brain tissue. The authors suggest that amylin and pramlintide enhance the removal of Aβ from the brain and its transfer into the blood, probably through their effects on cerebral vasculature, and amylin and pramlintide may represent a novel therapeutic avenue for the treatment of AD. However, more work is needed to determine whether amylin is a beneficial or contributory factor to AD (Yang and Song, 2013).

G. Amylin in Renal Physiology

The effects of amylin in the kidney have been overlooked in recent years but amylin has interesting effects on renal physiology. In the spontaneously hypertensive rat model, the density of amylin binding sites was greater before the development of hypertension, compared with control Wistar-Kyoto rats. As blood pressure increased with age in the spontaneously hypertensive rat model group, so too did the density of amylin binding sites (Wookey et al., 1997). Thus, amylin and its receptor(s) may contribute to the development or maintenance of hypertension (Wookey and Cooper, 1998). Amylin may also act as a growth factor in the kidney and may control water and sodium reabsorption (Harris et al., 1997; Wookey et al., 1998). In anesthetized rats, amylin infusion can also promote diuresis and natriuresis (Vine et al., 1998b).

VIII. Amylin Therapeutic Applications: Overview/Rationale

In this section, we consider the effects of amylin (and its analog pramlintide) in the context of diabetes and obesity, the two therapeutic areas that the biology we have described in sections I–VI gears it toward. We describe special features of amylin physiology in the context of preclinical and clinical studies that are important for its mechanism of action. In section X, we specifically describe the clinical trials conducted with amylin agonists in the context of overall trial endpoint and therapeutic suitability for diabetes or obesity.

A. Diabetes

1. Amylin Secretion in Diabetes. There is an absence of secretion of β-cell hormones in type 1 diabetes, whereas β-cell dysregulation in type 2 diabetes (depending on its severity) can range from mild to severe. Since insulin's discovery in 1922, treatment of type 1 and late-stage type 2 diabetes has consisted of exogenous insulin dosing in response to glucose measurements. The concept of using insulin to treat diabetes stems from replacing the loss of pancreatic β-cell hormone as it is missing or severely undersecreted in either type 1 diabetes or late-stage type 2 diabetes. Although insulin monotherapy has been a life-saving strategy for these insulin-deficient patients, even optimally controlled patients exhibit glycemic hypervariability in response to relatively modest perturbations in fuel balance. One of the main reasons that insulin therapy is not adequate in the treatment of diabetes is the fact that we now recognize that diabetes is not as simple as a β-cell failure disease or a monohormonal (insulin) disease. It is becoming more widely appreciated that adjunctive therapies that enable reductions in exogenous insulin are quite desirable. For example, in a percentage of patients with type 1 diabetes (approximately 12%–40%) (McGill et al., 2008), long-term insulin monotherapy is associated with development of metabolic syndrome. This is felt to be mediated by inducing a persistent state of hyperinsulinemia wherein exogenous insulin is circulating in blood at higher concentrations than is needed at target tissues. In turn, individuals that develop metabolic syndrome are at an increased risk for developing clinical microvascular and macrovascular complications and mortality. One rational candidate for adjunctive therapy with insulin would be its partner hormone amylin, which is also deficient in type 1 diabetes and is secreted from the pancreatic β-cell in a fixed molar ratio with insulin (Young, 2005d). One study investigated the role in humans of endogenous amylin to regulate β-cell function The investigators treated patients with the amylin receptor antagonist, AC253, generating data that support an endogenous role of amylin to regulate insulin under certain conditions and/or disease states (Mather et al., 2002).

2. Postprandial Hyperglucagonemia and Diabetes. As noted above, the optimal treatment of diabetes requires more than insulin replacement because of the involvement of multiple hormonal systems involved in maintaining tight glycemic control. Juxtaposed to insulin/amylin-containing pancreatic β-cells are α-cells that release glucagon to maintain blood glucose levels at the appropriate concentration (Young, 2005c). In individuals without diabetes, glucagon prevents hypoglycemia by causing the liver to convert stored glycogen into glucose, which is released into the bloodstream. As part of an elegant feedback system, glucagon also stimulates the release of insulin, which enables glucose to be taken up and used by insulin-dependent tissues and then insulin in turn also acts directly upon α-cells to suppress glucagon release. However, in patients with diabetes, α-cells are deprived of insulin signaling, leading to abnormally high glucagon concentrations (Young, 2005c). It is especially notable that during the postprandial period, not only is glucagon abnormally high, but it is paradoxically elevated in response to food intake, in turn further contributing to postprandial hyperglycemia. Although exogenous insulin replacement ultimately stimulates the α-cells to suppress glucagon, the concentrations of insulin required also stimulate the liver and muscle, resulting in a blockade in hepatic glucose production and increasing peripheral glucose disposal. Collectively, this hormonal milieu contributes to suboptimal glycemic control.

Glucagonostatic effects of amylin were first demonstrated in anesthetized rats through the use of an arginine tolerance test (because amino acids are a stimulus for glucagon release). These studies revealed that amylin's effects to suppress glucagon were quite potent (achieved at an EC_{50} of 18 pM, which is in the physiologic range for endogenously secreted amylin in rats) (Gedulin et al., 1997). Subsequent rodent studies confirmed a similar profile for the amylin analog, pramlintide. Interestingly, amylin antagonism with the amylin receptor antagonist AC187 increased glucagon levels approximately 2-fold, implying that amylinergic pathways may exert a tonic suppression upon glucagon secretion. Amylin's glucagonostatic properties do not appear to be modulated via direct effects of amylin upon α-cells because they are not evident in ex vivo (e.g., isolated-perfused pancreas) or in vitro studies (e.g., isolated pancreatic islets; Silvestre et al., 2001). These effects are most likely mediated via amylin activation of hindbrain receptors, leading to modulation of vagal efferent pathways to the pancreas, although this remains to be demonstrated experimentally. Finally, amylin's glucagonostatic effects are not evident in the face of insulin-induced hypoglycemia (Gedulin et al., 1997; Nauck et al., 2002). This phenomenon is referred to as a "hypoglycemic override mechanism" and is a desirable feature from a therapeutic perspective.

In patients with type 1 diabetes, postprandial secretion of glucagon is elevated and was inhibited by the administration of pramlintide (Nyholm et al., 1999; Levetan et al., 2003). Pramlintide also prevented the abnormal meal-related rise in glucagon in insulin-treated patients with type 1 diabetes (Fineman et al., 2002). However, although they were evident during normoglycemia, the glucagonostatic effects of pramlintide were not evident during insulin-induced hypoglycemia (Nyholm et al., 1996). Insulin-treated type 2 diabetes also presents with impaired suppression of glucagon secretion and hepatic glucose production,

which contribute to unsatisfactory glycemic control. In this patient group, pramlintide infusions were also demonstrated to reduce postprandial hyperglucagonemia (Nyholm et al., 1999). Hence, the effects of amylin on glucagon secretion identified in rodents appear to translate into the clinic to address an important metabolic derangement in diabetes.

3. Gastric Emptying and Diabetes. A second contributor to the regulation of postprandial glucose levels is the rate of gastric emptying. Vice versa, gastric emptying has also been reported to be regulated by glycemic status; it is slowed by hyperglycemia and accelerated by hypoglycemia (Horowitz and Fraser, 1994). As such, one potential contributing factor to increased postprandial glucose excursions in diabetes could be accelerated gastric emptying despite the clinical setting of hyperglycemia. Indeed, gastric emptying is accelerated in some rodent models of type 1 diabetes (e.g., BioBreeding rats; Plourde et al., 1993; Young et al., 1995) and streptozotocin-treated rats (Granneman and Stricker, 1984) and type 2 diabetes (e.g., Zucker Fatty rats; Green et al., 1997). Hypermotility has also been noted in clinical studies of individuals with type 1 diabetes (Nakanome et al., 1983) and to a greater extent type 2 diabetes, especially during its "early" stages (Jones et al., 1996). However, impaired motility has not been uniformly observed across clinical studies (reviewed in Ma et al., 2009). Other than patients with significant disease and development of significant gastroparesis, the majority of patients with diabetes exhibit hypergastric motility in the setting of hyperglycemia. Nevertheless, slowing gastric emptying represents one viable therapeutic strategy for reducing the rate of postmeal glucose excursions.

In preclinical studies, gastric emptying has been assessed by quantifying the rate of appearance after oral delivery of a variety of markers (e.g., phenol red, acetaminophen, radiolabeled nutrients). Most, but not all (Lutz et al., 1995b), studies suggested that anorectic doses of amylin also reduced gastric emptying in rats and humans (Young et al., 1995; Reidelberger et al., 2002; Young, 2005b); the effect seemed to be physiologically relevant because AC187 accelerated gastric emptying in rats (Gedulin et al., 2006). Amylin agonism dose-dependently slowed the rate of gastric emptying. The effects of rat amylin are quite potent (ED_{50} estimated at 0.42 nmol/kg) even in comparison with other peptidic regulators of nutrient uptake (e.g., GLP-1 and CCK have estimated ED_{50} values of 6.1 and 8.5 nmol/kg, respectively; Young et al., 1996a). Similar ED_{50} values in rodents have been obtained with pramlintide.

At high doses, rat amylin can fully inhibit gastric emptying in both normal and diabetic rats (Young et al., 1995). Amylin inhibition of gastric emptying is thought to be primarily centrally mediated. First, aspiration of the AP abolishes amylin's effects on gastric emptying (Young, 2005b). More recently, amylin inhibition of gastric emptying was demonstrated in rats with a subdiaphragmatic vagal deafferentation (e.g., in which vagal afferent fibers are destroyed but efferent fibers remain intact), supporting the hypothesis that amylin acts via hormonal mechanisms to stimulate the AP and transmits signals to the gut via efferent vagal fibers (Wickbom et al., 2008).

Because the lowest effective amylin doses to reduce meal size or to slow gastric emptying are comparable (Reidelberger et al., 2001), it was postulated that amylin's effect on gastric emptying contributes to amylin's anorectic effect under normal feeding conditions; however, the former effect does not seem to be necessary for the latter to occur. First, both effects are pharmacologically distinguishable (Young et al., 1996a; Young, 1997; Young and Denaro, 1998). Second, amylin also reduces eating under sham-feeding conditions, hence when gastric and postgastric feedback signals are eliminated (Asarian et al., 1998). Because higher doses were necessary to reduce sham feeding than real feeding (Asarian et al., 1998), amylin may synergistically interact with other negative feedback signals to reduce food intake; these may include gastric feedback (Reidelberger et al., 2001), CCK (Asarian et al., 1998; Bhavsar et al., 1998; Lutz et al., 2000), and potentially other signals.

The effects of pramlintide on gastric emptying have also been assessed in a number of clinical studies. Infusion of pramlintide delayed solid and liquid gastric emptying in patients with type 1 diabetes (Kong et al., 1997), and these effects were subsequently shown to be dose dependent and no longer evident after 4 hours (Kong et al., 1998). When the gastric emptying effects of pramlintide were compared in individuals with type 1 or type 2 diabetes, they were determined to be equally effective. Likewise, in volunteers without diabetes, pramlintide delayed gastric emptying without affecting small bowel or colonic transit (Samsom et al., 2000). Hence, the effects of amylin agonism on gastric emptying have been recapitulated in the clinic and are evident irrespective of disease state.

B. Obesity

The studies summarized in section V indicate that amylin physiologically contributes to the control of eating, energy expenditure, and body weight. On the basis of these actions, it could be hypothesized that reduced amylin secretion, action, or sensitivity may contribute to less satiation, increased meal sizes and overall eating, and lower energy expenditure, together eventually contributing to the development of obesity.

1. Amylin Secretion in the Obese State. Although regulation of amylin secretion has not been extensively evaluated in humans, there is some evidence for elevation of amylin in the obese state (Ludvik et al., 1991; Enoki et al., 1992; Hanabusa et al., 1992;

Blackard et al., 1994; Kautzky-Willer et al., 1994; Roth et al., 2010; Lee et al., 2011; Jacobsen et al., 2012). Plasma levels appear to reach up to approximately twice those achieved in nonobese subjects, but whether this results overall in sustained levels of amylin in excess of those that would be achieved postprandially is not clear. A study of amylin (and other hormones) in obese (n = 16) compared with control (n = 14) adolescent subjects showed elevated fasting amylin levels, alongside insulin resistance. In response to a test meal, amylin levels increased to a greater degree in the obese subjects compared with control subjects (Beglinger et al., 2014). A study by Jacobsen et al. (2012) evaluated hormonal changes in patients before and after bariatric surgery. In that study, there was an observed divergence of relationship between insulin and amylin in which insulin increased significantly postsurgery where amylin levels were unchanged. A study by Blackard et al. (1994) looked at the hormonal response in nine morbidly obese subjects via portal vein sampling at the time of bariatric surgery. Their data collaborated the concept of insulin and amylin being cosecreted; however, there was a lot of intersubject variation. Unfortunately, the clinical data to evaluate the secretion of amylin are still very limited and the available data should be used with caution due to the small number of experimental subjects in many of the studies.

On the other hand, several lines of preclinical research have produced hypothesis-generating data that could translate well with respect to clinical relevance. Previous data had indicated that baseline amylin levels may be higher in obesity (Pieber et al., 1994), but it was not clear whether the meal-contingent release of amylin was affected by high-fat diet exposure and whether the postprandial release pattern differed between obese and nonobese rats (Boyle et al., 2011). Because the meal-induced rise in amylin levels is thought to underlie the meal-ending effect of amylin and other satiation hormones, and because the relevance of higher fasting levels of satiation hormones for the control of eating is not clear, this experiment allowed us to test the hypothesis of whether a deficient rise in amylin secretion in response to a meal may contribute to overeating and ultimately the development of obesity. Using the well described diet-induced obese (DIO) rat model, we found that both high-fat diet exposure and obesity influenced the meal-induced amylin release (Boyle et al., 2011). Exposure to the high-fat diet affected baseline amylin levels because diet-resistant rats, which had a lower body weight than low-fat (chow)–fed rats, had elevated baseline amylin, and all rats on the high-fat diet demonstrated an earlier meal-induced rise in plasma amylin compared with the chow control group. Hence, in contrast with our hypothesis, we did not observe any indication of deficient amylin secretion in obesity or in the rats exposed to the high-fat diets, suggesting that deficient amylin secretion does not contribute to the development of obesity, at least under the conditions tested (Boyle et al., 2011).

In summary, although basal amylin levels may be slightly elevated, a meal-induced rise in plasma amylin is still observed in the obese state, indicating that there is no major impairment in secretion. However, more work is needed to determine whether there are more subtle differences in absolute amount of amylin released and whether there are long-term effects of diet on the patterns of amylin secretion.

2. Amylin Sensitivity in the Obese State. A well described phenomenon in obesity is the development of resistance to the eating-inhibitory effects of exogenous leptin and insulin, as well as to meal-related signals like CCK and GLP-1 or their agonists (Deacon and Ahrén, 2011; Zhou and Rui, 2013). Leptin insensitivity has typically been documented by reduced induction of pSTAT3, an intracellular signaling molecule that is instrumental in mediating leptin function (Wauman and Tavernier, 2011). The decreased pSTAT3 response in leptin target neurons correlates with a reduced eating-inhibitory effect of leptin. Obesity may also reduce insulin and leptin transport into the brain by affecting the blood–brain barrier permeability (Banks, 2006). Finally, hyperleptinemia itself, which develops as a consequence of reduced sensitivity to leptin in obesity, seems to further reduce the animals' sensitivity to the action of leptin (Zhang and Scarpace, 2006).

Similar to the question above, we recently investigated whether reduced sensitivity to amylin's effects on eating or energy expenditure may contribute to the development of obesity. In other words, we wanted to know whether amylin action may also be affected by obesity or the chronic intake of diets high in fat (Boyle et al., 2011). First, we found no evidence that rats that were chronically maintained on a high-fat diet would reduce the rats' sensitivity to acute amylin. The intake of a high-fat diet alone did not seem to markedly alter amylin sensitivity; despite a slight attenuation of the animals' response to acute amylin after many months on the high-fat diet, amylin sensitivity was not lost completely (Boyle et al., 2011). Second, although amylin transport across the blood–brain barrier has been described (Banks and Kastin, 1998), this transport may not be necessary for many AP-mediated central amylin actions because the AP is devoid of a blood–brain barrier. Hence, even if reduced amylin transport across the blood–brain barrier occurred in obesity (which has not been tested), the relevance of this phenomenon would probably be minor.

Third, we tested whether chronic increases in plasma amylin could be a cause of reduced amylin sensitivity in obese rats. Because hyperleptinemia seems to be required for the full development of leptin resistance in rats (Knight et al., 2010), and because increased circulating levels of CCK are implicated in

a reduced sensitivity to CCK (Covasa et al., 2001), we tested whether elevated circulating baseline amylin may decrease the sensitivity to acute exogenous amylin administration. However, we observed that amylin acutely decreased eating to a similar extent in all rats and regardless of circulating amylin levels (Boyle and Lutz, 2011; Boyle et al., 2011). Hence, at least under the experimental conditions of our study, a decrease in the sensitivity to the eating-inhibitory effect of acute amylin was not apparent and a down-regulation of amylin receptors or a decrease in postreceptor signaling after hyperamylinemia therefore appeared to be unlikely.

Finally, we tested whether exposure to highly palatable diets like Ensure nutrition shakes (Abbott Nutrition, Columbus, OH; Wielinga et al., 2010) acutely changed amylin sensitivity. We found that at least temporarily, acute exposure to palatable chocolate Ensure reduced the eating-inhibitory effect induced by acute central or peripheral amylin. However, high doses of central amylin were still active, suggesting that rats had not become completely amylin insensitive (Wielinga et al., 2010). In another study, amylin or salmon calcitonin may be less potent in models of reduced leptin sensitivity (or signaling) but not in DIO models. Salmon calcitonin could effectively inhibit food intake in DIO animals with functional leptin resistance (Eiden et al., 2002).

To summarize, reduced amylin sensitivity is observed only under some experimental conditions. In contrast with leptin resistance, where central leptin receptor function seems to be compromised, particularly in its presumed primary sites of action in the arcuate nucleus and VMH, amylin receptor function in the AP was similar in lean chow-fed or obese rats with diet-induced obesity. Hence, amylin insensitivity may not be caused by direct changes in amylin receptor function, but tests whether intracellular signaling systems like cGMP and pERK (Riediger et al., 2001; Potes et al., 2012) are affected by body adiposity or diet composition still need to be done. Although decreased leptin transport across the blood–brain barrier appears to play a role in leptin resistance (Banks, 2006), such an effect is unlikely to be of relevance for amylin. Finally, rats with elevated plasma amylin seem to be fully responsive to acute amylin injections. Overall, our results suggest that reduced amylin sensitivity may not be a prominent feature contributing to the development of obesity; however, there has been little investigation of this in human obesity.

3. Food Intake and Body Weight: Preclinical Studies in Diet-Induced Obese Rat Models. DIO-prone rats exhibit many characteristics of human obesity (increased fat mass, obesity-related disturbances such as dyslipidemia and hyperinsulinemia) and lack genetic disruption of key feeding-related central signaling pathways (Levin and Dunn-Meynell, 2000). Importantly, the anorexigenic effects of peripheral amylin, which were largely demonstrated in acute tests in lean animals, translated into meaningful weight loss in these models (Roth et al., 2006). In DIO rats, peripherally administered rat amylin (3–300 μg/kg per day) for 4 weeks reduced food intake and decreased body weight by 10%–14% at the highest doses tested. Pair-feeding studies revealed some important differences between amylin-induced weight loss compared with that induced by caloric restriction alone (Roth et al., 2006). Although food intake reduction was the predominant mode of action for overall weight loss, the composition of weight loss was notably different across treatment groups. In amylin-treated rats, weight loss was entirely attributable to reductions in fat mass, with relative preservation of lean mass. By contrast, pair-fed control animals experienced reductions in both fat and lean body mass. Amylin-induced weight loss was also demonstrated to not be associated with counter-regulatory decreases in energy expenditure typically associated with a reduced weight state. When amylin's weight- and fat-reducing properties were evaluated across a variety of nutritive states, they were determined to still be evident after a diet "lead-in" regimen (Roth et al., 2007a; Wielinga et al., 2010). Finally, amylin monotherapy remained similarly effective at reducing body weight irrespective of starting body weight; in other words, there does not appear to be "amylin resistance" to the weight-lowering actions of amylin with increasing obesity (reviewed in Lutz, 2012b).

At this point, it is important to mention that in many studies with rats or mice mentioned here or elsewhere in this review, amylin treatment resulted in a significant reduction in (vehicle-corrected) body weight compared with control animals; however, amylin-treated animals may still have exhibited a gain in absolute body weight, albeit less than in controls. One may, therefore, argue that this effect is not sufficient for a clinically relevant action. There is, however, a critical difference between rodents and humans in this respect—that is, most rat and mouse strains keep gaining body weight more or less throughout their entire life span. Furthermore, amylin-induced decreases in absolute body weight (true weight loss) have been reported in weight-stable rats (retired female breeders) (Roth et al., 2007a).

C. Identifying Amylin Agonist-Responsive Populations in Preclinical Models

With the arsenal of available therapies for metabolic diseases continuing to evolve, it is likely that subgroups of target populations will exhibit differential responsiveness to any given pharmacological agent. Just as large differences in the regulation of energy homeostasis exist between males and females, it is not unreasonable to assume that therapeutic modalities

will be more, or less, efficacious as concentrations of gonadal hormones fluctuate during development and aging. Sexually dimorphic effects are especially relevant with respect to neurohormone-based therapies for metabolic diseases, because pharmacological and neurobiological studies have convincingly demonstrated a role for estradiol in the central control of energy balance. These studies generally predict that the presence of estradiol enhances the anorexigenic properties of short-term signals of satiation (e.g., CCK) and long-term signals of adiposity (e.g., leptin), while concomitantly decreasing the potency of orexigenic signals (e.g., melanin concentrating hormone, neuropeptide Y, ghrelin; reviewed in Asarian and Geary, 2006; Brown and Clegg, 2010; Butera, 2010). We recently explored whether estradiol signaling affected the weight regulatory and metabolic effects of amylin agonism (see also Fig. 5) (Trevaskis et al., 2010c). We observed a surprising approximately 2-fold increase in amylin's weight-lowering efficacy in a state of estradiol deficiency [DIO ovariectomized (OVX) rat model] compared with sham-operated controls. Detailed metabolic studies revealed that amylin reversed the decline in energy expenditure and fat oxidation linked to an estradiol-deficient state via food intake–dependent (reduction in respiratory exchange ratio) and food intake–independent (maintenance of metabolic rate) mechanisms. Because postmenopausal women comprise a large majority of patients for whom weight loss agents are prescribed, the extent to which these preclinical observations translate into the clinical use of the amylin agonist pramlintide warrants further investigation. These studies should also comprise detailed mechanistic experiments that clarify the apparent paradox that while under chronic conditions, the lack of estradiol enhances amylin action, but exogenous and endogenous amylin were more effective when given acutely to OVX rats that received physiologic estrogen replacement therapy (Asarian et al., 2011).

Interestingly, an antidepressive and neurogenic profile of amylin agonism was also noted in these studies. First, amylin restored neurogenesis in the hippocampus of OVX rats and increased (approximately 2-fold) neurogenesis within the AP. These alterations were not evident in sham rats treated with amylin. Amylin-treated OVX rats also displayed decreased immobility (i.e., improvements in this depressed phenotype) compared with sham-operated controls in the forced swim test. These findings warrant further exploration but they are noteworthy because estradiol concentrations decrease in postmenopausal women, who make up a high percentage of the obese population, and incidences of depression are reported to be increasing during menopause (Asarian and Geary, 2006). To what extent amylin agonism may hold utility in neurodegenerative diseases remains to be elucidated. Collectively, these preclinical findings raise the intriguing possibility that the integrated mechanisms of amylin may improve metabolic and behavioral processes and represent an important area for future clinical research.

D. Next-Generation Drugs for the Amylin System

Pramlintide is a useful mimetic of human amylin but other amylin agonists with improved potency and pharmacokinetics may provide optimized therapeutics for the treatment of obesity. Several additional analogs of human amylin have been synthesized and investigated. The feasibility of orally administered amylin has also recently been explored. There are also alternative approaches that could be used, such as inhibition of amylin degradation.

1. Davalintide. Davalintide is an amylinomimetic that showed significantly enhanced potency, efficacy, duration of action, and pharmaceutical properties for producing weight loss in rodent models (Mack et al., 2010). Davalintide is a 32-amino-acid peptide that shares 49% identity to rat amylin and pramlintide, and it is a chimera of amylin and salmon calcitonin (Fig. 3). Davalintide displays similar in vitro binding affinity to rat amylin in a rat amylin/calcitonin receptor preparation (nucleus accumbens), 10-fold greater affinity compared with rat amylin at the human CGRP receptor (SK-N-MC cells), and 100-fold greater affinity than rat amylin at the rat calcitonin receptor (transfected HEK293 cells). Davalintide and amylin showed comparable potency to activate cAMP production in RINm5F cells (Mack et al., 2010). A direct comparison between the pharmacology of davalintide, salmon calcitonin, and amylin at defined calcitonin and amylin receptor subtypes would be informative to provide deeper understanding of the molecular properties of this peptide.

In satiated rats, davalintide significantly reduced food consumption up to 23 hours after injection. By contrast, at an equivalent dose, rat amylin significantly decreased food intake only from 1 to 3 and 5 to 6 hours after treatment. In DIO rats, sustained davalintide infusion dose-dependently decreased food intake, body weight, and fat mass to a greater extent than that observed with rat amylin. In general, davalintide exhibited greater duration of action, approximately 10-fold greater potency to reduce food intake, and 2-fold greater efficacy to reduce body weight compared with rat amylin in rats. With respect to effects on food preference, consumption of a high-fat (palatable) diet was significantly reduced by davalintide and amylin compared with control animals. Both davalintide and amylin reduce eating by an AP-mediated mechanism and both activate similar brain nuclei, with davalintide displaying an extended duration of c-Fos expression compared with amylin (8 versus 2 hours) (Mack et al., 2010). Interestingly, these effects exceed those

predicted by its pharmacokinetic profile [the half-life of davalintide (200 μg/kg s.c.) was 26 minutes]. Analyses of receptor binding kinetics showed limited dissociation from rat nucleus accumbens membranes (Mack et al., 2011). This is not surprising, given that it contains portions of the salmon calcitonin sequence; salmon calcitonin is well recognized as having a slow receptor dissociation rate from receptors (Hilton et al., 2000). These findings suggest that it may be possible to design amylinomimetics with a longer duration of action that would require fewer daily injections. Glucoregulatory actions of davalintide were also studied in rodents, and this amylinomimetic peptide displayed similar but more protracted actions versus rat amylin on glucose lowering and suppression of gastric emptying (Mack et al., 2011).

2. PEGylated or Glycosylated Amylin. Given the short half-life of amylin, another strategy to improve the molecule has been to improve its half-life through the addition of molecular scaffolds such as polyethylene glycol (PEG). A recent preparation has been described for the PEGylation of amylin (Guerreiro et al., 2013). Subcutaneous administration of PEGylated amylin in mice revealed the effectiveness of monoPEG-amylin and diPEG-amylin in reducing glycemia, with both compounds exhibiting prolonged action relative to unmodified amylin (Guerreiro et al., 2013). Further testing of these molecules is warranted. Several pharmaceutical companies have also been investigating improving the pharmaceutical properties of pramlintide via either PEGylation or adding albumin binding motifs (e.g., Amylin, Unigene, Novo Nordisk, and AstraZeneca) to develop a daily or weekly injection product (Hansen et al., 2007; Mehta et al., 2013). Another strategy was recently employed in which glycosylation of pramlintide was tested as a possible means of enhancing half-life. The synthetic glycopeptides that were generated had in vitro and in vivo activity, suggesting that this could be a useful approach for further investigation (Tomabechi et al., 2013; Kowalczyk et al., 2014). Hence, there remains strong interest in developing more patient-friendly amylin agonist strategies for treatment of metabolic diseases.

3. Oral Amylin Agonists. A few studies have shown proof of concept for oral administration of amylin agonists. Intravail (Aegis Therapeutics, LLC, San Diego, CA) is a broad class of chemically synthesizable transmucosal absorption enhancement agents. Pramlintide has been formulated in Intravail and after administration to exert the expected effects on energy balance and glycemic control in insulin-resistant male C57BLK/6-m db/db mice (Leinung and Grasso, 2012).

Recent preclinical findings suggest that orally administered salmon calcitonin may hold antidiabetic and weight-lowering potential. Salmon calcitonin was formulated in 5CNAC-sCT [*N*-(5-chlorosalicyloyl)-8-aminocaprylicacid]. When given orally, 5CNAC-sCT appears to be protected from upper digestive tract proteases. This enables the complex to access the higher pH environment in the upper small intestine intact and then dissociate enabling improved systemic absorption (reviewed in Karsdal et al., 2011). In DIO rats, oral gavage of this formulation reduced glucose and insulin area under the curve during an oral glucose tolerance test, and weight-lowering effects in various models have also been achieved (Feigh et al., 2011, 2012). These findings highlight the potential for oral delivery of amylin agonists rather than via multiple daily injections. It remains to be seen whether any of these formulations will be commercially feasible or pharmaceutically developable from a cost-of-goods and bioavailability perspective.

4. Inhibition of Amylin Degradation. Overall, several strategies have been investigated to search for novel amylin agonists that may possess more pharmaceutically and patient-friendly properties. However, the strategy of inhibiting degradation of endogenous amylin, as has been successfully used with the dipeptidyl peptidase-IV inhibitors to enhance plasma levels of endogenous active GLP-1 (Holst, 2004), has to date not been evaluated for amylin peptides. Amylin's action is relatively short lived because of its short half-life, and termination of amylin action seems to be mainly due to renal metabolism and excretion (Leckström et al., 1997; Vine et al., 1998a). Therefore, manipulation of its degradation may not be a useful pharmacological approach. Furthermore, the target plasma concentrations required to produce significant glucose-lowering or weight loss benefits in humans may be beyond that which can be achieved solely via inhibition of peptide degradation.

IX. Amylin Interactions: Physiology and Pharmacology

A. Amylin and Leptin

The most extensively investigated amylin-based combination is with the adipokine leptin (Fig. 6). Leptin is regarded as the prototypical long-term signal of energy balance; however, obese rodents and humans are largely nonresponsive to exogenous leptin administration. Early studies demonstrated that acute third ventricular leptin increased the eating-inhibitory effect of peripheral amylin in lean rats (Osto et al., 2007). Next, exogenous pharmacological amylin was shown to synergize with peripherally administered leptin to induce marked and sustained fat-specific body weight loss in leptin-resistant DIO rats (Roth et al., 2008a; Trevaskis et al., 2008). These findings suggested that amylin may help restore leptin responsiveness in obesity. The effects appear to be pharmacological in nature; in other words, both agonists need to be present for leptin's pharmacological effects to be

evident in obesity. DIO rats pretreated with amylin or with the combination of amylin and leptin did not maintain their weight loss when switched to a regimen of leptin monotherapy (Roth et al., 2008a; Turek et al., 2010). Studies exploring caloric restriction, surgery, and coadministration of other anorexigens suggest that amylin appears to be one of the best modalities for restoring leptin responsiveness. Caloric restriction alone does not appear to be sufficient to restore leptin responsiveness in preclinical models or obese human subjects. For example, rats pair fed and weight matched to an amylin-treated group did not lose additional weight when leptin was coadministered (Roth et al., 2008a).

In terms of other pharmacologies, doses of PYY(3-36) or an exenatide analog were selected that gave a similar magnitude of weight loss (6%) that was sufficient for amylin to restore leptin responsiveness (Roth et al., 2008a). In these studies, only additive to less than additive effects were observed. In line with these findings, leptin replacement alone or with exendin-4 failed to promote greater weight loss in weight-reduced DIO rats (Reidelberger et al., 2012). Although a leptin/exendin synergy has been reported in DIO mice, it was only evident after an initial body weight loss of 30% (Müller et al., 2012), far greater than the 6% required for amylin/leptin synergy. Mechanistic studies suggest that the presence of

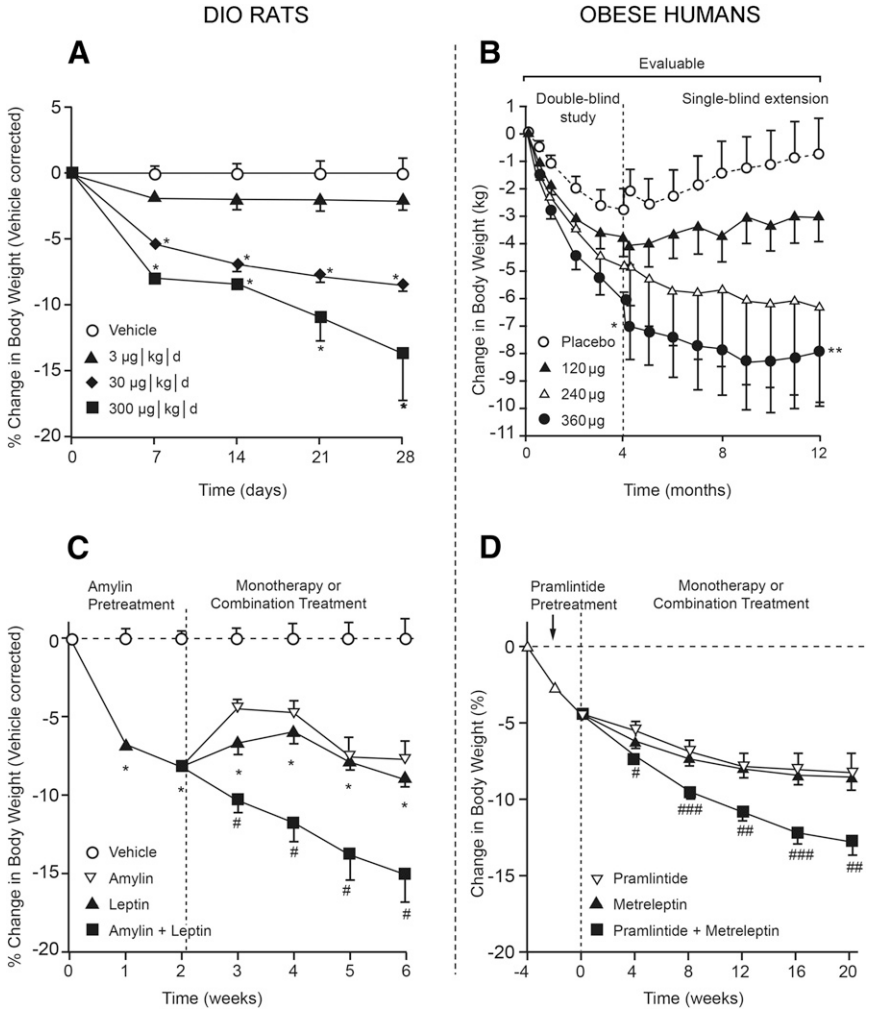

Fig. 6. (A) Effect of amylin on body weight in DIO rats. Change in body weight (percent vehicle corrected) of rats receiving amylin (3–300 μg/kg per day) for 4 weeks via subcutaneously implanted osmotic minipumps. Mean ± S.E.M. $^{*}P < 0.05$ versus vehicle. (B) Effect of pramlintide in obese subjects. Changes in body weight from baseline for placebo (n = 17), 120 μg pramlintide (n = 24), 240 μg pramlintide (n = 17), or 360 μg pramlintide (n = 21) twice daily (evaluable population). Mean ± S.E.M. $^{*}P < 0.05$; $^{**}P < 0.01$ versus placebo. (C) Effect of amylin, leptin, or amylin plus leptin on body weight in DIO rats. Change in body weight (percent vehicle corrected) of rats pretreated for 14 days with 100 μg/kg per day amylin and then maintained on 100 μg/kg per day amylin switched to either 500 μg/kg per day leptin monotherapy or 100 μg/kg per day amylin plus 500 μg/kg per day leptin combination therapy (n = 6–8/group). Compounds were delivered for 6 weeks via subcutaneously implanted osmotic minipumps. Mean ± S.E.M. $^{*}P < 0.05$ versus vehicle; $^{\#}P < 0.05$, compared with monotherapies. (D) Effect of metreleptin, pramlintide, or metreleptin plus pramlintide combination on body weight in obese subjects. Changes in body weight from enrollment (week 4) for subjects pretreated with pramlintide (titrated from 180 μg to 360 μg) twice and then treated with 360 μg pramlintide twice daily, 5 mg metreleptin twice daily, or pramlintide plus metreleptin combination treatment (evaluable population, n = 93). Mean ± S.E.M. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$, compared with monotherapies. This figure has been redrawn from the individual publications in which these data were presented. (A) contains data from Mack et al. (2010); (B) contains data from Smith et al. (2008); and (C and D) are from Roth et al. (2008a).

amylin "primes" the hypothalamus to respond to leptin by enhancing leptin receptor number and/or signaling capacity (for a recent review of the physiologic, neurobiological, and molecular mechanisms mediating these interactions, see Trevaskis et al., 2010b). This may be due to amylin increasing interleukin-6 expression (Le Foll et al., 2015). It was recently reported that leptin and amylin may also interact functionally in the VTA (Mietlicki-Baase et al., 2015).

B. Amylin and PYY(3-36)

PYY is a 36-amino-acid peptide hormone that is secreted from intestinal L cells after a meal and is cleaved by dipeptidyl peptidase-IV to generate PYY (3-36), the major circulating form (Grandt et al., 1994). Experiments in rodents have shown that PYY(3-36) inhibits food intake, reduces body weight gain, and increases utilization of fat stores for energy. A series of rodent studies examined the effects of combined administration of amylin and PYY(3-36). Overall, these studies revealed that amylin and PYY(3-36) together were able to elicit a greater reduction in 24-hour food intake after a single acute injection than either monotherapy without significantly changing locomotor activity. With sustained administration, the combination synergistically reduced food intake and additively reduced body weight in DIO-prone rats (Roth et al., 2007b).

C. Amylin and Cholecystokinin

CCK is a short-term satiation signal released from intestinal L cells after nutrient consumption. After its release, CCK is thought to exert a paracrine effect by its actions on vagal afferents that project to the NTS. CCK acutely synergizes with amylin to suppress food intake in lean mice (Bhavsar et al., 1998; Mollet et al., 2003a). To expand on these findings, sustained administration studies (7 days) assessed whether the combination of amylin and CCK would synergize for weight loss in DIO rats. As expected, amylin treatment induced significant body weight loss. CCK, although ineffective alone, significantly enhanced body weight loss when coadministered with higher doses of amylin. c-Fos activation was also assessed in various brain nuclei after a single intraperitoneal injection of amylin and/or CCK. Amylin and CCK additively increased c-Fos within the AP, predominantly in noradrenergic (e.g., DBH-containing) cells.

D. Amylin and Melanocortins

An important downstream regulator of leptin is the hypothalamic melanocortin (MC) system. Convergent lines of evidence underscore the importance of the melanocortin subtype-4 receptor (MC-4R) as a key regulator of feeding behavior. The impact of impaired MC signaling on amylin's efficacy has been evaluated in two species. Chow-fed overnight fasted agouti and wild-type mice respond similarly to an acute dose of amylin (Roth et al., 2006). However, MC-4R–knockout mice displayed quantitatively reduced responsiveness to salmon calcitonin at a dose range that was effective in chow-fed C57Bl/6J mice (overnight fasted) (Eiden et al., 2002), and $Mc4r^{K314X/K314X}$ rats were nonresponsive to an acute dose of amylin that was effective in wild-type rats. Overall, it is unclear whether amylin's acute food intake–lowering effects are MC-4R dependent (Roth et al., 2012). One explanation for these discrepant findings may be that these are two different models of MC dysregulation; agouti mice are a model of ectopic endogenous MC-3R/MC-4R antagonism (by agouti overexpression), whereas the $Mc4r^{K314X/K314X}$ rat is a receptor loss-of-function model. The methodological and procedural differences (e.g., species, diet composition, nutritive status) across these different studies may also play a role. Nevertheless, coadministration studies clearly demonstrate the antiobesity potential for combined agonism of these MC and amylinergic systems. Amylin coadministration enhanced the acute food intake–lowering effects of Ac-R[CEH-dF-RWC]-amide in mice and with repeated coadministration had durable, additive anorexigenic, weight-lowering, and fat-lowering effects in DIO rats (Roth et al., 2012).

E. Amylin and Glucagon-Like Peptide 1

Combined amylin and GLP-1 receptor agonism has also been assessed (Bello et al., 2010). This study evaluated the acute anorexigenic effects of coadministering the amylin/calcitonin receptor agonist salmon calcitonin alone and in combination with GLP-1 receptor agonist exenatide in a non–human primate model. Monkeys were maintained on a schedule of 6-hour daily access to food and multiple dose combinations were used. This experimental design enabled the authors to use response surface methodology and test for statistical additivity or synergy for suppression of food intake. Synergistic anorexigenic effects were noted during hours 1–4 and additive effects during hours 5 and 6. Behavioral observations suggested that the monkeys did not display malaise or nausea. After a washout period, monkeys received repeated daily injections of one of the dose combinations (0.56 μg/kg Exendin-4 + 0.32 μg/kg salmon calcitonin) for 5 days. During this phase of the study, sustained reductions in daily food intake (>70% from saline baseline) were noted for 5 days, suggesting that the additive/synergistic effects of the combination may be durable. Future repeated administration studies should include monotherapy arms to give a better sense for the potential additive/synergistic potential of this combination.

F. Amylin and Small Molecule Anorectics

Amylin has also been combined with various classes of small molecule anorectic agents that are either still approved for the short-term treatment of obesity

(phentermine), are withdrawn from market (sibutramine), or are in late stages of regulatory review (naltrexone/bupropion). These studies are reviewed below.

Phentermine is a β-phenethylaminic derivative that acts through monoaminergic systems (noradrenaline and dopamine) to decrease food intake and body weight. Sibutramine inhibits the reuptake of central noradrenaline and serotonin and stimulates thermogenesis in rodents. Mathematically additive food intake, weight-lowering, and fat-lowering effects were observed when amylin was combined with either phentermine or sibutramine. Detailed analyses of feeding patterns with amylin and phentermine suggest that these effects may be achieved by an approximately 2-fold increase in the satiety ratio compared with either agent alone (Roth et al., 2008c). In terms of off-target behavioral effects, amylin neither enhanced nor reduced the known actions of phentermine (which is an amphetamine derivate) to stimulate activity (Roth et al., 2008c).

Antagonism of opioid systems (e.g., with naltrexone) is another antiobesity strategy, and it is particularly effective when coadministered with dual inhibitors of dopamine and norepinephrine reuptake (e.g., bupropion). The interaction of amylin with naltrexone/bupropion on energy balance has recently been explored (Clapper et al., 2013). Wild-type and amylin knockout mice were similarly responsive to the food intake–lowering effects of either naltrexone (1 mg/kg s.c.) or bupropion (50 mg/kg s.c.), suggesting that they act independently of amylinergic systems and could interact additively when given in combination with amylin. To test this, DIO rats were treated (for 11 days) with vehicle, rat amylin (50 μg/kg per day s.c.), naltrexone/bupropion (1 and 20 mg/kg, respectively, by twice-daily s.c. injection), or their combination. Amylin plus naltrexone/bupropion combination therapy exerted additive effects to reduce cumulative food intake, body weight, and fat mass. In a separate study, the effects of amylin and naltrexone/bupropion administered at the same doses (for 14 days) were compared with a pair-fed group. Although the combination and pair-fed groups lost a similar amount of body weight, rats treated with the combination lost 68% more fat and better maintained their lean mass. These findings support the strategy of combined amylin agonism with opioid and catecholaminergic signaling systems for the treatment of obesity.

X. Amylin in Clinical Studies

This section covers many of the major clinical studies of amylin/pramlintide. In some places, we also describe relevant preclinical studies to aid in understanding how translatable these findings were to the clinic.

A. Pharmacology of Amylin Agonists

Pramlintide is a synthetic and equipotent analog of human amylin and is used as an adjunctive therapy to patients treated with mealtime insulin. It differs from the natural peptide by three amino acids; prolines were substituted at positions 25, 28, and 29 (Fig. 3). These substitutions were necessary to overcome several physicochemical properties that make human amylin unsuitable for pharmacologic delivery, including poor stability in solution and a propensity to aggregate and adhere to surfaces. Hence, amylin's biology and therapeutic potential in humans is almost exclusively assessed using pramlintide. By contrast, reports on the actions of amylin in rodents have been assessed using either rat amylin (which also possesses favorable pharmaceutical properties) or pramlintide. In general, the pharmacological and pharmacokinetic properties of pramlintide are identical to those of rat amylin, when the two peptides are compared in preclinical rodent models (Young et al., 1996b).

The pharmacokinetics of pramlintide in rats reflects a half-life of approximately 13 minutes after intravenous injection, a value identical to that of rat amylin (Young et al., 1996b). A similar profile is seen with intravenous injection of human amylin into normal human males (Clodi et al., 1998).With subcutaneous injection in rodents, half-lives for both rat amylin and pramlintide are in the range of 15–35 minutes, whereas bioavailability ranged between 25% and 40%. In normal humans, subcutaneous injections of pramlintide ranging from 300 to 10,000 μg produced half-life values varying from 26 to 42 minutes (Moyses et al., 1993). In subjects with type 1 and type 2 diabetes, subcutaneous doses ranging from 30 to 180 μg gave similar profiles, with maximal plasma concentrations achieved in approximately 20 minutes and a half-life of around 50 minutes (Colburn et al., 1996; Weyer et al., 2001). Two recent articles can be consulted for more information on the pharmacokinetics of pramlintide (Younk et al., 2011; Fang et al., 2013).

B. Clinical Studies Relating to Diabetes

Collectively, multiple clinical studies in insulin-using patients with type 1 and type 2 diabetes suggest that pramlintide addresses important deficits in the treatment of diabetes, and pramlintide in combination with insulin may provide a more physiologically balanced therapeutic approach (Younk et al., 2011). A 2011 review of the literature pertaining to the clinical data for the use of pramlintide in diabetes covers most of these studies in some depth (Younk et al., 2011). In brief, pramlintide treatment improved hemoglobin A1c, reduced postprandial glucose excursions relative to insulin therapy alone, enabled reductions in mealtime insulin dosing, inhibited postprandial glucagon secretion, and improved overall glycemic control. Although excess weight gain is associated with many antihyperglycemic therapies and can potentially induce further insulin resistance, pramlintide treatment

was associated with sustained and significant weight loss. This aspect was apparent without prescribed dietary/lifestyle interventions and is explored in more detail below. Nausea (mild to moderate) is the most frequently reported adverse event for pramlintide, yet the weight loss experienced with pramlintide is largely independent of any reported nausea.

Pramlintide was approved in the United States as an adjunct therapy to mealtime insulin. The subcutaneous dose of pramlintide is fixed with 60 μg for patients with type 1 diabetes and 120 μg for patients with type 2 diabetes, and patients are instructed to dose pramlintide 30 minutes before a meal (Janes et al., 1996; Center for Drug Evaluation and Research Approval Package for Application Number 21-332. Clinical Pharmacology and Biopharmaceutics Review. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/21-332_Symlin%20Injection_biopharmr.pdf). Given the fact that pramlintide is delivered as a separate injection to mealtime insulin, compliance for patients may be an issue. One study in patients with type 2 diabetes evaluated the addition of pramlintide versus mealtime insulin as an adjunct to basal insulin to reduce the injection burden and the clinical effect of pramlintide without mealtime insulin. The results showed that pramlintide was similar to rapid-acting mealtime insulin with glycemic reduction but with less hypoglycemia and no weight gain (Riddle et al., 2009).

In patients with type 1 diabetes, not only is compliance an issue, but these patients are also more prone to insulin-induced hypoglycemia. Few studies have looked at ways to make the combination easier and potentially safer for this population. One study showed that acute mixing of pramlintide with rapid-acting or basal insulin did not significantly alter the pharmacokinetics and clinical effectiveness of pramlintide; however, there are no long-term mixing studies to assess the stability of both molecules (Weyer et al., 2005). Insulin-induced hypoglycemia is the most serious complication when using pramlintide with mealtime insulin in the type 1 diabetes population. Anecdotally, clinicians have reported that dosing analog insulin before meals in conjunction with pramlintide leads to more hypoglycemia because patients underestimate the satiety effect of pramlintide and end up overdosing the mealtime insulin. Given this, some clinicians have started dosing mealtime insulin after the meal rather than before the meal. Although it has not yet been officially examined, we can see this trend with some of the clinical trial designs for patients with type 1 diabetes using pramlintide as an adjunct to mealtime insulin (Hassan and Heptulla, 2009).

Because more patients with type 1 diabetes are using pump therapy, investigators have looked at different ways of delivering the mealtime insulin dose in conjunction with pramlintide. Two separate studies have been conducted to evaluate how best to dose insulin in a patient using pump therapy, in conjunction with pramlintide. One study tested four different insulin delivery wave forms: standard, square wave, combination (standard shifting to square), and modified combination (standard combined with square 1 hour into the meal). In that study, it was found that the square and modified combination was the safest way to deliver insulin (King, 2010). Another study found that a square wave delivery for patients with type 1 diabetes near normal control was the best bolus approach, whereas with weight loss, there may need to be adjustments to total basal insulin dose to avoid hypoglycemia (King, 2009).

Amylin and insulin are colocated and cosecreted in a fixed molar ratio from the pancreatic β-cells; therefore, amylin is also secreted in a basal-bolus fashion. However, in clinical practice, insulin is often titrated for clinical effect, whereas pramlintide is delivered in a fixed dose and only at mealtime. Few studies have evaluated the effect of pramlintide delivered in a basal-bolus fashion. A 24-hour infusion study evaluating pramlintide with insulin basal-bolus infusion versus insulin infusion alone found that there was a significant decrease in postprandial glycemic control and robust glucagon suppression (Heptulla et al., 2009). Given the exploratory nature of this study, the pramlintide dose was calculated based on previous experience but had to be changed during the study. Another pilot study evaluated the pramlintide basal-bolus concept over a 16-week period. The basal pramlintide rate was 9 μg/h and the bolus dose was administered per label up to 60 μg/bolus at mealtime. The study showed significant glucose, insulin dose, and weight reduction but an increase in mild hypoglycemia (Huffman et al., 2009). A real-world pilot study with a small cohort of patients with type 1 diabetes (n = 5) and patients with type 2 diabetes (n = 5) with variable duration of diabetes and variable pramlintide to insulin ratios over a span of 1–5 years found that delivery of pramlintide in a separate pump in a basal-bolus fashion resulted in improved glycemic control, insulin dose reduction, and weight loss (Schorr and Ofan, 2012). Therefore, there may be some alternative dosing strategies that are possible for pramlintide in patients with diabetes, beyond current recommendations.

Given its physiologic actions and its clinical profile, pramlintide is an attractive candidate as an additional therapy to be included in the artificial pancreas in the treatment of type 1 diabetes. Excessive glycemic variability and hypoglycemia are major obstacles in the development of an artificial pancreas. A study evaluated dosing of 30 μg pramlintide bolus under a closed loop system and showed that pramlintide not only delayed the time to peak of postprandial hyperglycemia, but it also significantly reduced the glycemic excursion (Weinzimer et al., 2012). There are currently

no data to indicate the optimal pramlintide to insulin ratio. Most of the studies done thus far tested variable ratios in combination with a fixed dose bolus. At the time of this writing, there is an active pilot study being conducted by the Juvenile Diabetes Research Foundation to evaluate the optimal insulin to pramlintide ratio in the clinical setting (ClinicalTrials.gov identifier NCT01708044: http://www.clinicaltrials.gov/ct2/show/NCT01708044?term=pramlintide&rank=13). Although much work has been done, more studies are still needed to evaluate the role of pramlintide in an artificial pancreas.

C. Clinical Studies Relating to Food Intake, Satiation, and Weight Loss

The aforementioned observation that pramlintide reduced body weight in patients with insulin-treated diabetes, a patient population that generally suffers from weight gain due to insulin, pointed to its potential clinical utility as an antiobesity agent (Chapman et al., 2005; Aronne et al., 2007). In clinical studies in obese subjects, the weight-reducing effects of pramlintide emerged as significant, sustained, and dose dependent (Hollander et al., 2004; Smith et al., 2008; Singh-Franco et al., 2011). Interestingly, weight loss occurred despite the fact that subjects were instructed to maintain their usual diet and exercise routines. Progressive weight loss was also seen in those subjects who did not report nausea, providing further evidence that the weight-lowering effect of pramlintide is dissociable from the occurrence of nausea. The use of pramlintide alongside lifestyle intervention resulted in weight loss and enhanced long-term maintenance of weight loss in obese subjects.

Weight loss is likely to be linked with the food intake–lowering effects of pramlintide, which have been demonstrated by several clinical studies in obese subjects. A single injection of pramlintide 1 hour before a buffet meal decreased food intake by 16% compared with placebo (Smith et al., 2007). Pramlintide also elicited an increase in satiety scores, despite the consumption of less food. This indicates that less food was required to promote meal-related satiation. In other studies, pramlintide reduced total daily food intake (by 15%–20%) in obese subjects when measured both early during the study as well as after 6 weeks of administration (Chapman et al., 2005, 2007). Pramlintide was also shown to cause acute and sustained reductions in the intake of high-fat and high-sugar foods at a "fast-food challenge" and to improve perceived control of eating, as demonstrated by a 45% placebo-corrected reduction in binge eating scores (Smith et al., 2007). These findings suggest that the nuances of changes in feeding behavior (e.g., decreased meal size while concomitantly increasing satiation, reduced intake and preference for high-fat and high-sugar foods), many of which are observed in preclinical models with amylin, may also be recapitulated in the clinic with pramlintide.

Pramlintide was evaluated in conjunction with lifestyle intervention in a phase 2b dose-ranging proof-of-concept study (Smith et al., 2008). Subjects who entered the study were obese [body mass index [BMI] $\geq$ 30 and $\leq$ 50 kg/m^2 for at least 1 year] nondiabetic men and women aged 18–70 years with abdominal obesity [waist circumference >102 cm for men and >88 cm for women]. In this 4-month, double-blind, placebo-controlled, dose-ranging study, 411 obese subjects were randomized to receive pramlintide (six arms: 120, 240, and 360 μg twice daily and three times daily) or placebo in conjunction with a structured lifestyle intervention program geared toward weight loss. At the end of 4 months, 77% of the evaluable subjects (n = 270) opted to continue preexisting treatment during an 8-month single-blind extension with lifestyle intervention geared toward weight maintenance. At month 4, mean weight loss from baseline in the pramlintide arms ranged from 3.8 $\pm$ 0.7 to 6.1 $\pm$ 0.8 kg (2.8 $\pm$ 0.8 kg with placebo). By month 12, initial four-month weight loss was regained in the placebo group but was maintained in all but the 120 μg twice-daily group. Placebo-corrected weight loss with 120 μg three times daily and 360 μg twice daily averaged 3.2 $\pm$ 1.2 kg (3.1% $\pm$ 1.1% body weight) and 3.3 $\pm$ 1.1 kg (3.1% $\pm$ 1.0% body weight), respectively, at month 4 (both $P < 0.01$; 4-month evaluable n = 270) and 6.1 $\pm$ 2.1 kg (5.6% $\pm$ 2.1% body weight) and 7.2 $\pm$ 2.3 kg (6.8% $\pm$ 2.3% body weight), respectively, at month 12 (both $P < 0.01$; 12-month evaluable n = 146). At month 12, 40% and 43% of subjects treated with 120 μg three times daily and 360 μg twice daily, respectively, achieved $\geq$10% weight loss (versus 12% for placebo). Nausea was the most common adverse event with pramlintide in the 4-month study (9%–29% pramlintide versus 2% placebo) and it was generally mild to moderate and occurred in <10% of subjects during the extension. Collectively, these studies suggest that obese subjects treated with pramlintide alone or as an adjunct to lifestyle intervention can achieve weight loss and enhanced long-term maintenance of weight loss. Fig. 6 compares preclinical and clinical data for pramlintide-induced effects on body weight.

Davalintide has also undergone limited clinical investigation. Translational medicine studies revealed suppression of 24-hour food intake in obese humans treated with davalintide (Nicandro et al., 2008).

D. Clinical Combination Studies (Metreleptin and Small Molecules) Relating to Weight Loss

Obesity is a multifaceted disease. There are multiple and redundant pathways to avoid weight loss as a means of survival. Monotherapy for obesity, although initially effective, often only yields modest and unsustained weight loss. As with other chronic diseases such

as hypertension, cancer, and diabetes, combinatorial approaches have gained significant traction for the potential treatment of obesity (Kim et al., 2013). Analogous to diabetes, monotherapy-based approaches for obesity target only a single aspect of a multihormonal disease state. Although it was taken off the market for safety reasons, the history of fenfluramine/phentermine demonstrated that by targeting more than one single pathway in the control of weight, a robust and sustainable weight loss can be achieved. Accordingly, a series of translational research studies have demonstrated the potential utility of combined amylin agonism with small molecule anorexigenic agents (e.g., phentermine or sibutramine) or with an analog of the long-term adiposity signal leptin (e.g., metreleptin) that may be useful in overcoming these adaptations to promote meaningful weight loss. These preclinical studies are described in section IX. Some clinical investigation has also been conducted.

As discussed above, the neurohormonal control of body weight involves a complex interplay between long-term adiposity signals (e.g., leptin), and short-term satiation signals (e.g., amylin). We have discussed that in DIO rodents, amylin/leptin combination treatment led to marked, synergistic, fat-specific weight loss (Fig. 6). To evaluate the weight-lowering effect of combined amylin/leptin agonism (with pramlintide/metreleptin) in human obesity, a 24-week, randomized, double-blind, active drug–controlled, proof-of-concept study was conducted in obese or overweight subjects (n = 177; 63% female; aged 39 ± 8 years; BMI 32.0 ± 2.1 kg/m^2; weight 93.3 ± 13.2 kg; mean ± S.D.) (Ravussin et al., 2009). The study started with a 4-week lead-in period for the titration of pramlintide and dietary intervention. After the initial lead-in period with pramlintide (180 μg twice daily for 2 weeks, 360 μg twice daily thereafter) and diet (40% calorie deficit), subjects achieving 2%–8% weight loss were randomized 1:2:2 to 20 weeks of treatment with metreleptin (5 mg twice daily), pramlintide (360 μg twice daily), or pramlintide/metreleptin (360 μg/5 mg twice daily). Combination treatment with pramlintide/metreleptin led to significantly greater weight loss from enrollment to week 20 (−12.7% ± 0.9%; least-squares mean ± S.E.) than treatment with pramlintide (−8.4% ± 0.9%; $P < 0.001$) or metreleptin (−8.2% ± 1.3%; $P < 0.01$) alone (evaluable, n = 93). The greater reduction in body weight was significant as early as week 4, and weight loss continued throughout the study, without evidence of a plateau even at the 24-week end point. The most common adverse events with pramlintide/metreleptin were consistent with the two molecules studied and were mainly injection site events and nausea, which were mostly mild to moderate and decreased over time. These results further support the concept that combination treatment that targets separate physiologic pathways is a viable approach for the induction of clinically meaningful weight loss.

A functional magnetic resonance imaging study recently compared activation after the administration of metreleptin with the combination of metreleptin with pramlintide. Pramlintide significantly potentiated leptin activation in mesolimbic brain regions such as the hippocampus and amygdala (Klopfenstein et al., 2010). To what extent these findings explain the weight loss synergy between amylin and leptin agonism remains to be determined.

Based on the preclinical finding of amylin in combination with small molecule oral medications like phentermine or sibutramine, a proof-of-concept phase 2 human clinical study was conducted (Aronne et al., 2010). This study was a randomized placebo-controlled trial, and it examined the safety and efficacy of the amylin analog pramlintide alone or in combination with either phentermine or sibutramine. All patients enrolled in the study also received lifestyle intervention. After a 1-week placebo lead-in period, 244 obese or overweight subjects without diabetes (88% female; 41 ± 11 years; BMI 37.7 ± 5.4 kg/m^2; weight 103 ± 19 kg; mean ± S.D.) received placebo (subcutaneously three times a day), pramlintide (120 μg subcutaneously three times a day), pramlintide (120 μg subcutaneously three times a day) plus oral sibutramine (10 mg every day before noon), or pramlintide (120 μg subcutaneously three times a day) plus oral phentermine (37.5 mg every day before noon) for 24 weeks. Treatment was single blind for subjects receiving subcutaneous medication only and open label for subjects in the combination arms. Weight loss achieved at week 24 with either combination treatment was greater than with pramlintide alone or placebo ($P < 0.001$; −11.1% ± 1.1% with pramlintide plus sibutramine, −11.3% ± 0.9% with pramlintide plus phentermine, −3.7% ± 0.7% with pramlintide, −2.2% ± 0.7% with placebo; mean ± S.E.). Given that phentermine and sibutramine all have potential to raise blood pressure or heart rate, this cardiovascular safety parameter was of particular importance in a population at higher risk for cardiovascular events. Elevations in heart rate and diastolic blood pressure from baseline were demonstrated with both pramlintide plus sibutramine (3.1 ± 1.2 beats/min, $P < 0.05$; 2.7 ± 0.9 mm Hg, $P < 0.01$) and pramlintide plus phentermine (4.5 ± 1.3 beats/min, $P < 0.01$; 3.5 ± 1.2 mm Hg, $P < 0.001$) using 24-hour ambulatory monitoring. However, the majority of subjects receiving these treatments remained within normal blood pressure ranges. These results validated the preclinical observation that amylin agonism combined with another pathway that targets weight management may have additive or synergistic effects.

As we have stated before, obesity is a complex disease and due to the evolutionary need for energy preservation, there are multiple pathways to prevent weight loss. Monotherapy in general does not elicit the amount of weight loss patients and health care

providers are looking for. The most robust clinical data have come from combination therapies. However, the development of small molecule obesity drugs has had major safety issues in the past. The combination of phentermine and fenfluramine was taken off the market due to cardiac valvulopathy issues from fenfluramine. The drug rimonabant that leverages the cannabinoid pathway was also taken off the market for suicidal risks. The most recent obesity therapy to be taken off the market was sibutramine due to potential increase in cardiovascular events. Despite these setbacks, the U.S. Food and Drug Administration recently approved another selective serotonin agonist like fenfluramine called lorcaserin. As a monotherapy, it elicits a modest weight loss of around 2 kg (Fidler et al., 2011). Another therapy that was recently approved was a combination of phentermine and topiramate. Despite combining different pathways, the weight loss was also modest and the highest dose did elicit the most significant side effects (Gadde et al., 2011). Despite new approvals, the obesity treatment landscape is still very limited due to potential safety concerns (Kim et al., 2013). The recent U.S. Food and Drug Administration approval of the GLP-1 receptor agonist liraglutide for obesity (Saxenda; Novo Nordisk Inc., Plainsboro, NJ) provides a new opportunity for trialling combinations of pramlintide and liraglutide in patients (Ladenheim, 2015). The attraction of leveraging the physiologic pathway of amylin agonist is that it can potentially deliver a more clinically meaningful weight loss while minimizing the off-target adverse events.

XI. Concluding Remarks

This review summarizes the tremendous increase in knowledge about the physiology and pharmacology of the pancreatic hormone amylin. As a member of the CGRP family of peptides, amylin shares a large number of biologic effects with calcitonin, CGRP, and adrenomedullin. However, the effects of amylin in particular in the control of energy homeostasis and its current acceptance in the scientific community as a short-term satiation factor clearly stand out and have been the basis of many important physiologic and clinical findings. Landmark studies in this area have shown the following: 1) the first report that amylin decreases eating in rats after central and peripheral administration (Chance et al., 1991; Morley et al., 1993); 2) that the caudal hindbrain and in particular the AP is critically involved in the effects of amylin on eating (Lutz et al., 1998c); 3) that amylin interacts with other endogenous regulators of eating, in particular with leptin, and that it overcomes leptin resistance in obesity (Osto et al., 2007; Roth et al., 2008a); 4) that amylin alone or in combination produces a clinically relevant weight loss in humans (Smith et al., 2008; Ravussin et al., 2009); and 5) some of amylin's effects, specifically amylin's action to regulate the rate of nutrient appearance in the blood, via slowing gastric emptying and reducing postprandial glucagon secretion, have already been translated into amylin-based pharmacotherapy that has been introduced for many years into clinical practice for the treatment of diabetes mellitus.

Amylin was originally discovered as the main component of islet amyloid deposition in patients with type 2 diabetes. It soon became clear that amylin is a physiologic secretion product of pancreatic β-cells and that it is cosecreted with insulin. An important breakthrough and surprising finding was the characterization of the amylin receptor as a two-component entity consisting of the calcitonin core receptor that requires the coexpression of one of several RAMPs to yield a specific amylin receptor (Christopoulos et al., 1999; Muff et al., 1999). Although this concept is not unique to the receptors of the CGRP family, it was unexpected at the time of the discovery of the amylin receptor. More than 20 years of research also let amylin emerge as an important physiologic regulator of meal size and perhaps of body weight. The brain structures involved in this effect have been well defined and the available data indicate that the amylin signaling system seems to remain active in obesity [i.e., under conditions when other signaling systems (e.g., leptin) become defective]. The progress in preclinical development was translated into the use of the amylin analog pramlintide as an adjunct therapy to insulin for patients with type 1 and type 2 diabetes, and the weight-reducing potential of this approach compared with insulin alone formed the basis for numerous clinical trials utilizing amylin analogs and combination therapies to combat obesity. Importantly, amylin is one of the few established gut/pancreatic hormones that has demonstrated durable, safe, tolerable, and clinically meaningful weight loss in humans. Few drugs are currently approved for the treatment of obesity and these drugs typically produce weight loss of approximately 4%–7%, which is often below the expectations of both patients and physicians and leads to underutilization of these therapies. Available weight loss agents and compounds in development are primarily small molecule oral medications that alter neurotransmitter levels. Because regulation of body weight appears to be governed by complex interactions in the brain between numerous centrally acting neurotransmitters, central neuropeptides, and peptide hormones arising from the periphery, combinations of agents targeted at distinct regulatory pathways may produce additive or synergistic effects on weight loss. In previously described translational research studies, combinations of amylin physiology with other hormones such as leptin have revealed intriguing pharmacological synergies where amylin appears to overcome the acquired resistance

against the actions of leptin associated with weight gain. Combination of amylin physiology with neurotransmitter pathways such as phentermine and sibutramine also yielded clinically significant weight loss by targeting different pathways of weight control. As demonstrated in both preclinical and clinical studies, amylin receptor agonism has emerged as a key component of an integrated neurohormonal therapeutic approach for weight reduction and maintenance that harnesses synergies among several naturally occurring signals. Hence, an amylin agonist has developed into a promising candidate for the pharmacological intervention against obesity.

Future efforts should focus on deepening our understanding of amylin physiology with respect to the mechanisms of amylin action in brainstem neurons but also in other brain areas regulating reward behavior. The detailed basis of the functional interaction of amylin with other hormones, such as convergence of signaling pathways, needs to be explored because this may open new, more targeted treatment options against obesity and other disorders. The particular amylin receptor pharmacology with its specific structure as a two-component entity may offer additional possibilities for direct manipulation of the amylin signaling system with minimal side effects; the receptor subtype needs to be identified for each amylin action. Finally, other potentially therapeutic effects of amylin need to be explored in more detail because they may form the basis for an extended use of amylin-based pharmacotherapy for a large number of disorders. Recent data have highlighted potential roles in AD and for amylin as an antipsychotic. Further characterization, clinical development, and subsequent commercial availability of longer-acting (e.g., once-weekly administration) amylin analogs should enhance the acceptance of this important therapeutic target for treatment of diseases including and beyond diabetes and obesity.

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References

Abedini A and Schmidt AM (2013) Mechanisms of islet amyloidosis toxicity in type 2 diabetes. *FEBS Lett* **587**:1119–1127.

Adler BL, Yarchoan M, Hwang HM, Louneva N, Blair JA, Palm R, Smith MA, Lee HG, Arnold SE, and Casadesus G (2014) Neuroprotective effects of the amylin analogue pramlintide on Alzheimer's disease pathogenesis and cognition. *Neurobiol Aging* **35**:793–801.

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M, Peters JA, and Harmar AJ; CGTP Collaborators (2013) The Concise Guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. *Br J Pharmacol* **170**: 1459–1581.

Andreassen KV, Hjuler ST, Furness SG, Sexton PM, Christopoulos A, Nosjean O, Karsdal MA, and Henriksen K (2014) Prolonged calcitonin receptor signaling by salmon, but not human calcitonin, reveals ligand bias. *PLoS ONE* **9**:e92042.

Archbold JK, Flanagan JU, Watkins HA, Gingell JJ, and Hay DL (2011) Structural insights into RAMP modification of secretin family G protein-coupled receptors: implications for drug development. *Trends Pharmacol Sci* **32**:591–600.

Armour SL, Foord S, Kenakin T, and Chen WJ (1999) Pharmacological characterization of receptor-activity-modifying proteins (RAMPs) and the human calcitonin receptor. *J Pharmacol Toxicol Methods* **42**:217–224.

Arnelo U, Permert J, Adrian TE, Larsson J, Westermark P, and Reidelberger RD (1996) Chronic infusion of islet amyloid polypeptide causes anorexia in rats. *Am J Physiol* **271**:R1654–R1659.

Aronne L, Fujioka K, Aroda V, Chen K, Halseth A, Kesty NC, Burns C, Lush CW, and Weyer C (2007) Progressive reduction in body weight after treatment with the amylin analog pramlintide in obese subjects: a phase 2, randomized, placebo-controlled, dose-escalation study. *J Clin Endocrinol Metab* **92**:2977–2983.

Aronne LJ, Halseth AE, Burns CM, Miller S, and Shen LZ (2010) Enhanced weight loss following coadministration of pramlintide with sibutramine or phentermine in a multicenter trial. *Obesity (Silver Spring)* **18**:1739–1746.

Asarian L, Boyle CN, and Lutz TA (2011) Estradiol (E2) increases the acute eating-inhibitory effect of amylin in ovariectomized (OVX) rats. *Appetite* **57** (Supplement 1):S2.

Asarian L, Eckel LA, and Geary N (1998) Behaviorally specific inhibition of sham feeding by amylin. *Peptides* **19**:1711–1718.

Asarian L and Geary N (2006) Modulation of appetite by gonadal steroid hormones. *Philos Trans R Soc Lond B Biol Sci* **361**:1251–1263.

Asmar M, Bache M, Knop FK, Madsbad S, and Holst JJ (2010) Do the actions of glucagon-like peptide-1 on gastric emptying, appetite, and food intake involve release of amylin in humans? *J Clin Endocrinol Metab* **95**:2367–2375.

Bailey RJ, Walker CS, Ferner AH, Loomes KM, Prijic G, Halim A, Whiting L, Phillips ARJ, and Hay DL (2012) Pharmacological characterization of rat amylin receptors: implications for the identification of amylin receptor subtypes. *Br J Pharmacol* **166**:151–167.

Baisley SK, Bremer QZ, Bakshi VP, and Baldo BA (2014) Antipsychotic-like actions of the satiety peptide, amylin, in ventral striatal regions marked by overlapping calcitonin receptor and RAMP-1 gene expression. *J Neurosci* **34**:4318–4325.

Baldo BA and Kelley AE (2001) Amylin infusion into rat nucleus accumbens potently depresses motor activity and ingestive behavior. *Am J Physiol Regul Integr Comp Physiol* **281**:R1232–R1242.

Banks WA (2006) The blood-brain barrier as a regulatory interface in the gut-brain axes. *Physiol Behav* **89**:472–476.

Banks WA and Kastin AJ (1998) Differential permeability of the blood-brain barrier to two pancreatic peptides: insulin and amylin. *Peptides* **19**:883–889.

Banks WA, Kastin AJ, Maness LM, Huang W, and Jaspan JB (1995) Permeability of the blood-brain barrier to amylin. *Life Sci* **57**:1993–2001.

Barrachina MD, Martínez V, Wang L, Wei JY, and Taché Y (1997) Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice. *Proc Natl Acad Sci USA* **94**:10455–10460.

Barth SW, Riediger T, Lutz TA, and Rechkemmer G (2004) Peripheral amylin activates circumventricular organs expressing calcitonin receptor a/b subtypes and receptor-activity modifying proteins in the rat. *Brain Res* **997**:97–102.

Beaumont K, Kenney MA, Young AA, and Rink TJ (1993) High affinity amylin binding sites in rat brain. *Mol Pharmacol* **44**:493–497.

Becskei C, Grabler V, Edwards GL, Riediger T, and Lutz TA (2007) Lesion of the lateral parabrachial nucleus attenuates the anorectic effect of peripheral amylin and CCK. *Brain Res* **1162**:76–84.

Becskei C, Riediger T, Zünd D, Wookey P, and Lutz TA (2004) Immunohistochemical mapping of calcitonin receptors in the adult rat brain. *Brain Res* **1030**:221–233.

Beglinger S, Meyer-Gerspach AC, Graf S, Zumsteg U, Drewe J, Beglinger C, and Gutzwiller JP (2014) Effect of a test meal on meal responses of satiation hormones and their association to insulin resistance in obese adolescents. *Obesity (Silver Spring)* **22**:2047–2052.

Bell D and McDermott BJ (1995) Activity of amylin at CGRP1-preferring receptors coupled to positive contractile response in rat ventricular cardiomyocytes. *Regul Pept* **60**:125–133.

Bello NT, Kemm MH, Ofeldt EM, and Moran TH (2010) Dose combinations of exendin-4 and salmon calcitonin produce additive and synergistic reductions in food intake in nonhuman primates. *Am J Physiol Regul Integr Comp Physiol* **299**: R945–R952.

Bhavsar S, Watkins J, and Young A (1998) Synergy between amylin and cholecystokinin for inhibition of food intake in mice. *Physiol Behav* **64**:557–561.

Bhogal R, Smith DM, and Bloom SR (1992) Investigation and characterization of binding sites for islet amyloid polypeptide in rat membranes. *Endocrinology* **130**: 906–913.

Blackard WG, Clore JN, and Kellum JM (1994) Amylin/insulin secretory ratios in morbidly obese man: inverse relationship with glucose disappearance rate. *J Clin Endocrinol Metab* **78**:1257–1260.

Bomberger JM, Parameswaran N, Hall CS, Aiyar N, and Spielman WS (2005a) Novel function for receptor activity-modifying proteins (RAMPs) in post-endocytic receptor trafficking. *J Biol Chem* **280**:9297–9307.

Bomberger JM, Spielman WS, Hall CS, Weinman EJ, and Parameswaran N (2005b) Receptor activity-modifying protein (RAMP) isoform-specific regulation of adrenomedullin receptor trafficking by NHERF-1. *J Biol Chem* **280**:23926–23935.

Booe JM, Walker CS, Barwell J, Kuteyi G, Simms J, Bill RM, Harris PW, Brimble MA, Poyner DR, and Hay DL, et al. (2015) Structural basis for receptor activity-modifying protein-dependent selective peptide recognition by a G protein-coupled receptor. *Mol Cell*, in press.

Bouali SM, Wimalawansa SJ, and Jolicoeur FB (1995) In vivo central actions of rat amylin. *Regul Pept* **56**:167–174.

Bouizar Z, Rostène WH, and Milhaud G (1987) Down-regulation of rat kidney calcitonin receptors by salmon calcitonin infusion evidenced by autoradiography. *Proc Natl Acad Sci USA* **84**:5125–5128.
Boyle CN and Lutz TA (2011) Amylinergic control of food intake in lean and obese rodents. *Physiol Behav* **105**:129–137.
Boyle CN, Rossier MM, and Lutz TA (2010) Diet-induced obesity, hyperamylinemia and amylin sensitivity. *Appetite* **54**:636.
Boyle CN, Rossier MM, and Lutz TA (2011) Influence of high-fat feeding, diet-induced obesity, and hyperamylinemia on the sensitivity to acute amylin. *Physiol Behav* **104**:20–28.
Brown LM and Clegg DJ (2010) Central effects of estradiol in the regulation of food intake, body weight, and adiposity. *J Steroid Biochem Mol Biol* **122**:65–73.
Butera PC (2010) Estradiol and the control of food intake. *Physiol Behav* **99**:175–180.
Butler AE, Jang J, Gurlo T, Carty MD, Soeller WC, and Butler PC (2004) Diabetes due to a progressive defect in beta-cell mass in rats transgenic for human islet amyloid polypeptide (HIP Rat): a new model for type 2 diabetes. *Diabetes* **53**: 1509–1516.
Castagné V, Moser PC, and Porsolt RD (2009) Preclinical behavioral models for predicting antipsychotic activity. *Adv Pharmacol* **57**:381–418.
Chance WT, Balasubramaniam A, Zhang FS, Wimalawansa SJ, and Fischer JE (1991) Anorexia following the intrahypothalamic administration of amylin. *Brain Res* **539**:352–354.
Chapman I, Parker B, Doran S, Feinle-Bisset C, Wishart J, Lush CW, Chen K, Lacerte C, Burns C, and McKay R, et al. (2007) Low-dose pramlintide reduced food intake and meal duration in healthy, normal-weight subjects. *Obesity (Silver Spring)* **15**:1179–1186.
Chapman I, Parker B, Doran S, Feinle-Bisset C, Wishart J, Strobel S, Wang Y, Burns C, Lush C, and Weyer C, et al. (2005) Effect of pramlintide on satiety and food intake in obese subjects and subjects with type 2 diabetes. *Diabetologia* **48**: 838–848.
Christopoulos A, Christopoulos G, Morfis M, Udawela M, Laburthe M, Couvineau A, Kuwasako K, Tilakaratne N, and Sexton PM (2003) Novel receptor partners and function of receptor activity-modifying proteins. *J Biol Chem* **278**:3293–3297.
Christopoulos G, Paxinos G, Huang XF, Beaumont K, Toga AW, and Sexton PM (1995) Comparative distribution of receptors for amylin and the related peptides calcitonin gene related peptide and calcitonin in rat and monkey brain. *Can J Physiol Pharmacol* **73**:1037–1041.
Christopoulos G, Perry KJ, Morfis M, Tilakaratne N, Gao Y, Fraser NJ, Main MJ, Foord SM, and Sexton PM (1999) Multiple amylin receptors arise from receptor activity-modifying protein interaction with the calcitonin receptor gene product. *Mol Pharmacol* **56**:235–242.
Clapper JR, Athanacio J, Wittmer C, Griffin PS, D'Souza L, Parkes DG, and Roth JD (2013) Effects of amylin and bupropion/naltrexone on food intake and body weight are interactive in rodent models. *Eur J Pharmacol* **698**:292–298.
Clodi M, Thomaseth K, Pacini G, Hermann K, Kautzky-Willer A, Waldhüsl W, Prager R, and Ludvik B (1998) Distribution and kinetics of amylin in humans. *Am J Physiol* **274**:E903–E908.
Colburn WA, Gottlieb AB, Koda J, and Kolterman OG (1996) Pharmacokinetics and pharmacodynamics of AC137 (25,28,29 tripro-amylin, human) after intravenous bolus and infusion doses in patients with insulin-dependent diabetes. *J Clin Pharmacol* **36**:13–24.
Cooper GJ, Willis AC, Clark A, Turner RC, Sim RB, and Reid KB (1987) Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. *Proc Natl Acad Sci USA* **84**:8628–8632.
Cooper GJS, Leighton B, Dimitriadis GD, Parry-Billings M, Kowalchuk JM, Howland K, Rothbard JB, Willis AC, and Reid KBM (1988) Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. *Proc Natl Acad Sci USA* **85**:7763–7766.
Cornish J, Callon KE, King AR, Cooper GJ, and Reid IR (1998) Systemic administration of amylin increases bone mass, linear growth, and adiposity in adult male mice. *Am J Physiol* **275**:E694–E699.
Covasa M, Marcuson JK, and Ritter RC (2001) Diminished satiation in rats exposed to elevated levels of endogenous or exogenous cholecystokinin. *Am J Physiol Regul Integr Comp Physiol* **280**:R331–R337.
Crawley JN and Beinfeld MC (1983) Rapid development of tolerance to the behavioural actions of cholecystokinin. *Nature* **302**:703–706.
Dackor R, Fritz-Six K, Smithies O, and Caron K (2007) Receptor activity-modifying proteins 2 and 3 have distinct physiological functions from embryogenesis to old age. *J Biol Chem* **282**:18094–18099.
Dacquin R, Davey RA, Laplace C, Levasseur R, Morris HA, Goldring SR, Gebre-Medhin S, Galson DL, Zajac JD, and Karsenty G (2004) Amylin inhibits bone resorption while the calcitonin receptor controls bone formation in vivo. *J Cell Biol* **164**:509–514.
Davey RA, Turner AG, McManus JF, Chiu WS, Tjahyono F, Moore AJ, Atkins GJ, Anderson PH, Ma C, and Glatt V, et al. (2008) Calcitonin receptor plays a physiological role to protect against hypercalcemia in mice. *J Bone Miner Res* **23**: 1182–1193.
Deacon CF and Ahrén B (2011) Physiology of incretins in health and disease. *Rev Diabet Stud* **8**:293–306.
Dhillo WS, Small CJ, Jethwa PH, Russell SH, Gardiner JV, Bewick GA, Seth A, Murphy KG, Ghatei MA, and Bloom SR (2003) Paraventricular nucleus administration of calcitonin gene-related peptide inhibits food intake and stimulates the hypothalamo-pituitary-adrenal axis. *Endocrinology* **144**:1420–1425.
Dobolyi A (2009) Central amylin expression and its induction in rat dams. *J Neurochem* **111**:1490–1500.
Edvinsson L and Warfvinge K (2013) CGRP receptor antagonism and migraine therapy. *Curr Protein Pept Sci* **14**:386–392.
Eftekhari S and Edvinsson L (2011) Calcitonin gene-related peptide (CGRP) and its receptor components in human and rat spinal trigeminal nucleus and spinal cord at C1-level. *BMC Neurosci* **12**:112.
Eiden S, Daniel C, Steinbrueck A, Schmidt I, and Simon E (2002) Salmon calcitonin - a potent inhibitor of food intake in states of impaired leptin signalling in laboratory rodents. *J Physiol* **541**:1041–1048.
Enoki S, Mitsukawa T, Takemura J, Nakazato M, Aburaya J, Toshimori H, and Matsukara S (1992) Plasma islet amyloid polypeptide levels in obesity, impaired glucose tolerance and non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract* **15**:97–102.
Fang J, Landersdorfer CB, Cirincione B, and Jusko WJ (2013) Study reanalysis using a mechanism-based pharmacokinetic/pharmacodynamic model of pramlintide in subjects with type 1 diabetes. *AAPS J* **15**:15–29.
Feigh M, Andreassen KV, Neutzsky-Wulff AV, Petersen ST, Hansen C, Bay-Jensen AC, Henriksen JE, Beck-Nielsen H, Christiansen C, and Henriksen K, et al. (2012) Oral salmon calcitonin attenuates hyperglycaemia and preserves pancreatic beta-cell area and function in Zucker diabetic fatty rats. *Br J Pharmacol* **167**:151–163.
Feigh M, Henriksen K, Andreassen KV, Hansen C, Henriksen JE, Beck-Nielsen H, Christiansen C, and Karsdal MA (2011) A novel oral form of salmon calcitonin improves glucose homeostasis and reduces body weight in diet-induced obese rats. *Diabetes Obes Metab* **13**:911–920.
Fernandes-Santos C, Zhang Z, Morgan DA, Guo DF, Russo AF, and Rahmouni K (2013) Amylin acts in the central nervous system to increase sympathetic nerve activity. *Endocrinology* **154**:2481–2488.
Fidler MC, Sanchez M, Raether B, Weissman NJ, Smith SR, Shanahan WR, and Anderson CM; BLOSSOM Clinical Trial Group (2011) A one-year randomized trial of lorcaserin for weight loss in obese and overweight adults: the BLOSSOM trial. *J Clin Endocrinol Metab* **96**:3067–3077.
Fineman MS, Koda JE, Shen LZ, Strobel SA, Maggs DG, Weyer C, and Kolterman OG (2002) The human amylin analog, pramlintide, corrects postprandial hyperglucagonemia in patients with type 1 diabetes. *Metabolism* **51**:636–641.
Flood JF and Morley JE (1992) Differential effects of amylin on memory processing using peripheral and central routes of administration. *Peptides* **13**:577–580.
Fry M, Hoyda TD, and Ferguson AV (2007) Making sense of it: roles of the sensory circumventricular organs in feeding and regulation of energy homeostasis. *Exp Biol Med (Maywood)* **232**:14–26.
Fukuda T, Hirai Y, Maezawa H, Kitagawa Y, and Funahashi M (2013) Electrophysiologically identified presynaptic mechanisms underlying amylinergic modulation of area postrema neuronal excitability in rat brain slices. *Brain Res* **1494**: 9–16.
Gadde KM, Allison DB, Ryan DH, Peterson CA, Troupin B, Schwiers ML, and Day WW (2011) Effects of low-dose, controlled-release, phentermine plus topiramate combination on weight and associated comorbidities in overweight and obese adults (CONQUER): a randomised, placebo-controlled, phase 3 trial. *Lancet* **377**: 1341–1352.
Geary N (2005) A new way of looking at eating. *Am J Physiol Regul Integr Comp Physiol* **288**:R1444–R1446.
Gebre-Medhin S, Mulder H, Pekny M, Westermark G, Törnell J, Westermark P, Sundler F, Ahrén B, and Betsholtz C (1998a) Increased insulin secretion and glucose tolerance in mice lacking islet amyloid polypeptide (amylin). *Biochem Biophys Res Commun* **250**:271–277.
Gebre-Medhin S, Mulder H, Zhang Y, Sundler F, and Betsholtz C (1998b) Reduced nociceptive behavior in islet amyloid polypeptide (amylin) knockout mice. *Brain Res Mol Brain Res* **63**:180–183.
Gedulin BR, Jodka CM, Herrmann K, and Young AA (2006) Role of endogenous amylin in glucagon secretion and gastric emptying in rats demonstrated with the selective antagonist, AC187. *Regul Pept* **137**:121–127.
Gedulin BR, Rink TJ, and Young AA (1997) Dose-response for glucagonostatic effect of amylin in rats. *Metabolism* **46**:67–70.
Gedulin BR and Young AA (1998) Hypoglycemia overrides amylin-mediated regulation of gastric emptying in rats. *Diabetes* **47**:93–97.
Gingell JJ, Burns ER, and Hay DL (2014) Activity of pramlintide, rat and human amylin but not Aβ1-42 at human amylin receptors. *Endocrinology* **155**:21–26.
Gingell JJ, Qi T, Bailey RJ, and Hay DL (2010) A key role for tryptophan 84 in receptor activity-modifying protein 1 in the amylin 1 receptor. *Peptides* **31**: 1400–1404.
Gloy VL, Lutz TA, Langhans W, Geary N, and Hillebrand JJ (2010) Basal plasma levels of insulin, leptin, ghrelin, and amylin do not signal adiposity in rats recovering from forced overweight. *Endocrinology* **151**:4280–4288.
Grabler V and Lutz TA (2004) Chronic infusion of the amylin antagonist AC 187 increases feeding in Zucker fa/fa rats but not in lean controls. *Physiol Behav* **81**: 481–488.
Grandt D, Schimiczek M, Beglinger C, Layer P, Goebell H, Eysselein VE, and Reeve JR Jr (1994) Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1-36 and PYY 3-36. *Regul Pept* **51**:151–159.
Granneman JG and Stricker EM (1984) Food intake and gastric emptying in rats with streptozotocin-induced diabetes. *Am J Physiol* **247**:R1054–R1061.
Green GM, Guan D, Schwartz JG, and Phillips WT (1997) Accelerated gastric emptying of glucose in Zucker type 2 diabetic rats: role in postprandial hyperglycaemia. *Diabetologia* **40**:136–142.
Guerreiro LH, Guterres MF, Melo-Ferreira B, Erthal LC, Rosa MdaS, Lourenço D, Tinoco P, and Lima LM (2013) Preparation and characterization of PEGylated amylin. *AAPS PharmSciTech* **14**:1083–1097.
Gutierrez-Rojas I, Lozano D, Nuche-Berenguer B, Moreno P, Acitores A, Ramos-Alvarez I, Rovira A, Novials A, Martin-Crespo E, Villanueva-Penacarrillo ML, et al. (2013) Amylin exerts osteogenic actions with different efficacy depending on the diabetic status. *Mol Cell Endocrinol* **365**:309–315.
Halford JC, Wanninayake SC, and Blundell JE (1998) Behavioral satiety sequence (BSS) for the diagnosis of drug action on food intake. *Pharmacol Biochem Behav* **61**: 159–168.
Hanabusa T, Kubo K, Oki C, Nakano Y, Okai K, Sanke T, and Nanjo K (1992) Islet amyloid polypeptide (IAPP) secretion from islet cells and its plasma concentration

in patients with non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract* **15**:89–96.
Hansen TK, Schaffer L, and Lau J (2007) inventors, Novo Nordisk A/S, assignee. Amylin derivatives. US Patent 20090099085. 2007 Mar 15.
Harikumar KG, Simms J, Christopoulos G, Sexton PM, and Miller LJ (2009) Molecular basis of association of receptor activity-modifying protein 3 with the family B G protein-coupled secretin receptor. *Biochemistry* **48**:11773–11785.
Harris PJ, Cooper ME, Hiranyachattada S, Berka JL, Kelly DJ, Nobes M, and Wookey PJ (1997) Amylin stimulates proximal tubular sodium transport and cell proliferation in the rat kidney. *Am J Physiol* **272**:F13–F21.
Hassan K and Heptulla RA (2009) Reducing postprandial hyperglycemia with adjuvant premeal pramlintide and postmeal insulin in children with type 1 diabetes mellitus. *Pediatr Diabetes* **10**:264–268.
Hay DL, Christopoulos G, Christopoulos A, Poyner DR, and Sexton PM (2005) Pharmacological discrimination of calcitonin receptor: receptor activity-modifying protein complexes. *Mol Pharmacol* **67**:1655–1665.
Hay DL, Harris PW, Kowalczyk R, Brimble MA, Rathbone DL, Barwell J, Conner AC, and Poyner DR (2014) Structure-activity relationships of the N-terminus of calcitonin gene-related peptide: key roles of alanine-5 and threonine-6 in receptor activation. *Br J Pharmacol* **171**:415–426.
Hay DL, Poyner DR, and Quirion R; International Union of Pharmacology (2008) International Union of Pharmacology. LXIX. Status of the calcitonin gene-related peptide subtype 2 receptor. *Pharmacol Rev* **60**:143–145.
Heptulla RA, Rodriguez LM, Mason KJ, and Haymond MW (2009) Twenty-four-hour simultaneous subcutaneous Basal-bolus administration of insulin and amylin in adolescents with type 1 diabetes decreases postprandial hyperglycemia. *J Clin Endocrinol Metab* **94**:1608–1611.
Hilton JM, Chai SY, and Sexton PM (1995) In vitro autoradiographic localization of the calcitonin receptor isoforms, C1a and C1b, in rat brain. *Neuroscience* **69**: 1223–1237.
Hilton JM, Dowton M, Houssami S, and Sexton PM (2000) Identification of key components in the irreversibility of salmon calcitonin binding to calcitonin receptors. *J Endocrinol* **166**:213–226.
Hollander P, Maggs DG, Ruggles JA, Fineman M, Shen L, Kolterman OG, and Weyer C (2004) Effect of pramlintide on weight in overweight and obese insulin-treated type 2 diabetes patients. *Obes Res* **12**:661–668.
Holst JJ (2004) Treatment of type 2 diabetes mellitus with agonists of the GLP-1 receptor or DPP-IV inhibitors. *Expert Opin Emerg Drugs* **9**:155–166.
Horcajada-Molteni MN, Chanteranne B, Lebecque P, Davicco MJ, Coxam V, Young A, and Barlet JP (2001) Amylin and bone metabolism in streptozotocin-induced diabetic rats. *J Bone Miner Res* **16**:958–965
Horcajada-Molteni MN, Davicco MJ, Lebecque P, Coxam V, Young AA, and Barlet JP (2000) Amylin inhibits ovariectomy-induced bone loss in rats. *J Endocrinol* **165**: 663–668.
Horowitz M and Fraser R (1994) Disordered gastric motor function in diabetes mellitus. *Diabetologia* **37**:543–551.
Hoyda TD, Smith PM, and Ferguson AV (2009) Gastrointestinal hormone actions in the central regulation of energy metabolism: potential sensory roles for the circumventricular organs. *Int J Obes (Lond)* **33** (Suppl 1):S16–S21.
Huang X, Yang J, Chang JK, and Dun NJ (2010) Amylin suppresses acetic acid-induced visceral pain and spinal c-fos expression in the mouse. *Neuroscience* **165**: 1429–1438.
Huffman DM, McLean GW, and Seagrove MA (2009) Continuous subcutaneous pramlintide infusion therapy in patients with type 1 diabetes: observations from a pilot study. *Endocr Pract* **15**:689–695.
Hwang JJ, Chan JL, Ntali G, Malkova D, and Mantzoros CS (2008) Leptin does not directly regulate the pancreatic hormones amylin and pancreatic polypeptide: interventional studies in humans. *Diabetes Care* **31**:945–951.
Isaksson B, Wang F, Permert J, Olsson M, Fruin B, Herrington MK, Enochsson L, Erlanson-Albertsson C, and Arnelo U (2005) Chronically administered islet amyloid polypeptide in rats serves as an adiposity inhibitor and regulates energy homeostasis. *Pancreatology* **5**:29–36.
Jacobsen SH, Olesen SC, Dirksen C, Jørgensen NB, Bojsen-Møller KN, Kielgast U, Worm D, Almdal T, Naver LS, and Hvolris LE, et al. (2012) Changes in gastrointestinal hormone responses, insulin sensitivity, and beta-cell function within 2 weeks after gastric bypass in non-diabetic subjects. *Obes Surg* **22**:1084–1096.
Jagger C, Chambers T, and Pondel M (2000) Transgenic mice reveal novel sites of calcitonin receptor gene expression during development. *Biochem Biophys Res Commun* **274**:124–129.
Janes S, Gaeta L, Beaumont K, Beeley N, and Rink T (1996) The selection of pramlintide for clinical evaluation (Abstract). *Diabetes* **45** (Suppl 2):235A.
Jones KL, Horowitz M, Carney BI, Wishart JM, Guha S, and Green L (1996) Gastric emptying in early noninsulin-dependent diabetes mellitus. *J Nucl Med* **37**: 1643–1648.
Kadmiel M, Fritz-Six K, and Caron K (2012) Understanding RAMPs through genetically engineered mouse models. *Adv Exp Med Biol* **744**:49–60.
Kadmiel M, Fritz-Six K, Pacharne S, Richards GO, Li M, Skerry TM, and Caron KM (2011) Research resource: Haploinsufficiency of receptor activity-modifying protein-2 (RAMP2) causes reduced fertility, hyperprolactinemia, skeletal abnormalities, and endocrine dysfunction in mice. *Mol Endocrinol* **25**:1244–1253.
Kanatsuka A, Makino H, Ohsawa H, Tokuyama Y, Yamaguchi T, Yoshida S, and Adachi M (1989) Secretion of islet amyloid polypeptide in response to glucose. *FEBS Lett* **259**:199–201.
Karsdal MA, Henriksen K, Bay-Jensen AC, Molloy B, Arnold M, John MR, Byrjalsen I, Azria M, Riis BJ, and Qvist P, et al. (2011) Lessons learned from the development of oral calcitonin: the first tablet formulation of a protein in phase III clinical trials. *J Clin Pharmacol* **51**:460–471.
Kautzky-Willer A, Thomaseth K, Pacini G, Clodi M, Ludvik B, Streli C, Waldhäusl W, and Prager R (1994) Role of islet amyloid polypeptide secretion in insulin-resistant humans. *Diabetologia* **37**:188–194.
Kaygisiz Z, Ozden H, Erkasap N, Koken T, Gunduz T, Ikizler M, and Kural T (2010) Positive inotropic, positive chronotropic and coronary vasodilatory effects of rat amylin: mechanisms of amylin-induced positive inotropy. *Acta Physiol Hung* **97**: 362–374.
Keller J, Catala-Lehnen P, Huebner AK, Jeschke A, Heckt T, Lueth A, Krause M, Koehne T, Albers J, and Schulze J, et al. (2014) Calcitonin controls bone formation by inhibiting the release of sphingosine 1-phosphate from osteoclasts. *Nat Commun* **5**:5215.
Kelley AE (1999) Functional specificity of ventral striatal compartments in appetitive behaviors. *Ann N Y Acad Sci* **877**:71–90.
Kim GW, Lin JE, Blomain ES, and Waldman SA (2013) New advances in models and strategies for developing anti-obesity drugs. *Expert Opin Drug Discov* **8**:655–671.
King AB (2009) Minimal reduction in insulin dosage with pramlintide therapy when pretreatment near-normal glycemia is established and square-wave meal bolus is used. *Endocr Pract* **15**:229–233.
King AB (2010) Comparison of the post-meal glucose response to different insulin bolus waveforms in insulin pump- and pre-meal pramlintide-treated type 1 diabetes patients. *Diabetes Technol Ther* **12**:105–108.
Klopfenstein BK, Krisky C, Rooney W, and Purnell JQ (2010) Amygdala activation by leptin predicts food intake in lean men. *Obesity (Silver Spring)* **18** (Suppl 1):76.
Knight ZA, Hannan KS, Greenberg ML, and Friedman JM (2010) Hyperleptinemia is required for the development of leptin resistance. *PLoS ONE* **5**:e11376.
Kong MF, King P, Macdonald IA, Stubbs TA, Perkins AC, Blackshaw PE, Moyses C, and Tattersall RB (1997) Infusion of pramlintide, a human amylin analogue, delays gastric emptying in men with IDDM. *Diabetologia* **40**:82–88.
Kong MF, Stubbs TA, King P, Macdonald IA, Lambourne JE, Blackshaw PE, Perkins AC, and Tattersall RB (1998) The effect of single doses of pramlintide on gastric emptying of two meals in men with IDDM. *Diabetologia* **41**:577–583.
Kowalczyk R, Brimble MA, Tomabechi Y, Fairbanks AJ, Fletcher M, and Hay DL (2014) Convergent chemoenzymatic synthesis of a library of glycosylated analogues of pramlintide: structure-activity relationships for amylin receptor agonism. *Org Biomol Chem* **12**:8142–8151.
Le Foll C, Johnson MD, Dunn-Meynell AA, Boyle CN, Lutz TA, and Levin BE (2015) Amylin-induced central IL-6 production enhances ventromedial hypothalamic leptin signaling. *Diabetes* **64**:1621–1631.
Ladenheim EE (2015) Liraglutide and obesity: a review of the data so far. *Drug Des Devel Ther* **9**:1867–1875.
Leckström A, Björklund K, Permert J, Larsson R, and Westermark P (1997) Renal elimination of islet amyloid polypeptide. *Biochem Biophys Res Commun* **239**: 265–268.
Lee HJ, Choe YH, Lee JH, Sohn YB, Kim SJ, Park SW, Son JS, Kim SW, and Jin DK (2011) Delayed response of amylin levels after an oral glucose challenge in children with Prader-Willi syndrome. *Yonsei Med J* **52**:257–262.
Leinung MC and Grasso P (2012) [D-Leu-4]-OB3, a synthetic peptide amide with leptin-like activity, augments the effects of orally delivered exenatide and pramlintide acetate on energy balance and glycemic control in insulin-resistant male C57BLK/6-m db/db mice. *Regul Pept* **179**:33–38.
Lenhart PM, Broselid S, Barrick CJ, Leeb-Lundberg LMF, and Caron KM (2013) G-protein-coupled receptor 30 interacts with receptor activity-modifying protein 3 and confers sex-dependent cardioprotection. *J Mol Endocrinol* **51**:191–202.
Levetan C, Want LL, Weyer C, Strobel SA, Crean J, Wang Y, Maggs DG, Kolterman OG, Chandran M, and Mudaliar SR, et al. (2003) Impact of pramlintide on glucose fluctuations and postprandial glucose, glucagon, and triglyceride excursions among patients with type 1 diabetes intensively treated with insulin pumps. *Diabetes Care* **26**:1–8.
Levin BE and Dunn-Meynell AA (2000) Defense of body weight against chronic caloric restriction in obesity-prone and -resistant rats. *Am J Physiol Regul Integr Comp Physiol* **278**:R231–R237.
Ludvik B, Lell B, Hartter E, Schnack C, and Prager R (1991) Decrease of stimulated amylin release precedes impairment of insulin secretion in type II diabetes. *Diabetes* **40**:1615–1619.
Lupien JR and Young AA (1993) No measurable effect of amylin on lipolysis in either white or brown isolated adipocytes from rats. *Diabetes Nutr Metab* **6**:13–18.
Luttrell LM (2006) Transmembrane signaling by G protein-coupled receptors. *Methods Mol Biol* **332**:3–49.
Lutz TA (2006) Amylinergic control of food intake. *Physiol Behav* **89**:465–471.
Lutz TA (2010) The role of amylin in the control of energy homeostasis. *Am J Physiol Regul Integr Comp Physiol* **298**:R1475–R1484.
Lutz TA (2012a) Control of energy homeostasis by amylin. *Cell Mol Life Sci* **69**: 1947–1965.
Lutz TA (2012b) Effects of amylin on eating and adiposity. *Handb Exp Pharmacol* **209**:231–250.
Lutz TA, Althaus J, Rossi R, and Scharrer E (1998a) Anorectic effect of amylin is not transmitted by capsaicin-sensitive nerve fibers. *Am J Physiol* **274**:R1777–R1782.
Lutz TA, Del Prete E, and Scharrer E (1994) Reduction of food intake in rats by intraperitoneal injection of low doses of amylin. *Physiol Behav* **55**:891–895.
Lutz TA, Del Prete E, and Scharrer E (1995a) Subdiaphragmatic vagotomy does not influence the anorectic effect of amylin. *Peptides* **16**:457–462.
Lutz TA and Geary N (2008) Gastrointestinal factors in appetite and food research - animal research, in Appetite and Food Intake: Behavioral and Physiological Consideration (Harris R, ed) pp 163–186, CRC Press, Boca Raton, FL.
Lutz TA, Geary N, Szabady MM, Del Prete E, and Scharrer E (1995b) Amylin decreases meal size in rats. *Physiol Behav* **58**:1197–1202.
Lutz TA, Mollet A, Rushing PA, Riediger T, and Scharrer E (2001) The anorectic effect of a chronic peripheral infusion of amylin is abolished in area postrema/nucleus of the solitary tract (AP/NTS) lesioned rats. *Int J Obes Relat Metab Disord* **25**:1005–1011.
Lutz TA, Rossi R, Althaus J, Del Prete E, and Scharrer E (1997) Evidence for a physiological role of central calcitonin gene-related peptide (CGRP) receptors in the control of food intake in rats. *Neurosci Lett* **230**:159–162.

Lutz TA, Rossi R, Althaus J, Del Prete E, and Scharrer E (1998b) Amylin reduces food intake more potently than calcitonin gene-related peptide (CGRP) when injected into the lateral brain ventricle in rats. *Peptides* **19**:1533–1540.

Lutz TA, Senn M, Althaus J, Del Prete E, Ehrensperger F, and Scharrer E (1998c) Lesion of the area postrema/nucleus of the solitary tract (AP/NTS) attenuates the anorectic effects of amylin and calcitonin gene-related peptide (CGRP) in rats. *Peptides* **19**:309–317.

Lutz TA, Tschudy S, Rushing PA, and Scharrer E (2000) Attenuation of the anorectic effects of cholecystokinin and bombesin by the specific amylin antagonist AC 253. *Physiol Behav* **70**:533–536.

Ma J, Rayner CK, Jones KL, and Horowitz M (2009) Insulin secretion in healthy subjects and patients with Type 2 diabetes—role of the gastrointestinal tract. *Best Pract Res Clin Endocrinol Metab* **23**:413–424.

Mack C, Wilson J, Athanacio J, Reynolds J, Laugero K, Guss S, Vu C, Roth J, and Parkes D (2007) Pharmacological actions of the peptide hormone amylin in the long-term regulation of food intake, food preference, and body weight. *Am J Physiol Regul Integr Comp Physiol* **293**:R1855–R1863.

Mack CM, Smith PA, Athanacio JR, Xu K, Wilson JK, Reynolds JM, Jodka CM, Lu MGW, and Parkes DG (2011) Glucoregulatory effects and prolonged duration of action of davalintide: a novel amylinomimetic peptide. *Diabetes Obes Metab* **13**: 1105–1113.

Mack CM, Soares CJ, Wilson JK, Athanacio JR, Turek VF, Trevaskis JL, Roth JD, Smith PA, Gedulin B, and Jodka CM, et al. (2010) Davalintide (AC2307), a novel amylin-mimetic peptide: enhanced pharmacological properties over native amylin to reduce food intake and body weight. *Int J Obes (Lond)* **34**:385–395.

Mather KJ, Paradisi G, Leaming R, Hook G, Steinberg HO, Fineberg N, Hanley R, and Baron AD (2002) Role of amylin in insulin secretion and action in humans: antagonist studies across the spectrum of insulin sensitivity. *Diabetes Metab Res Rev* **18**:118–126.

McGill M, Molyneaux L, Twigg SM, and Yue DK (2008) The metabolic syndrome in type 1 diabetes: does it exist and does it matter? *J Diabetes Complications* **22**:18–23.

McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, and Foord SM (1998) RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature* **393**:333–339.

Mehta NM, Sturmer A, and Stern W, and Gilligan JP (2013) inventors, Unigene Laboratories, assignee. Treatment for obesity. US Patent 8378067. 2013 Feb 19.

Mietlicki-Baase EG, Olivos DR, Jeffrey BA, and Hayes MR (2015) Cooperative interaction between leptin and amylin signaling in the ventral tegmental area for the control of food intake. *Am J Physiol Endocrinol Metab* DOI: 10.1152/ajpendo.00087.2015 [published ahead of print].

Mietlicki-Baase EG, Rupprecht LE, Olivos DR, Zimmer DJ, Alter MD, Pierce RC, Schmidt HD, and Hayes MR (2013) Amylin receptor signaling in the ventral tegmental area is physiologically relevant for the control of food intake. *Neuropsychopharmacology* **38**:1685–1697.

Mimee A, Smith PM, and Ferguson AV (2013) Circumventricular organs: targets for integration of circulating fluid and energy balance signals? *Physiol Behav* **121**: 96–102.

Mollet A, Gilg S, Riediger T, and Lutz TA (2004) Infusion of the amylin antagonist AC 187 into the area postrema increases food intake in rats. *Physiol Behav* **81**: 149–155.

Mollet A, Meier S, Grabler V, Gilg S, Scharrer E, and Lutz TA (2003a) Endogenous amylin contributes to the anorectic effects of cholecystokinin and bombesin. *Peptides* **24**:91–98.

Mollet A, Meier S, Riediger T, and Lutz TA (2003b) Histamine H1 receptors in the ventromedial hypothalamus mediate the anorectic action of the pancreatic hormone amylin. *Peptides* **24**:155–158.

Moon HS, Chamberland JP, Diakopoulos KN, Fiorenza CG, Ziemke F, Schneider B, and Mantzoros CS (2011) Leptin and amylin act in an additive manner to activate overlapping signaling pathways in peripheral tissues: in vitro and ex vivo studies in humans. *Diabetes Care* **34**:132–138.

Morfis M, Tilakaratne N, Furness SG, Christopoulos G, Werry TD, Christopoulos A, and Sexton PM (2008) Receptor activity-modifying proteins differentially modulate the G protein-coupling efficiency of amylin receptors. *Endocrinology* **149**: 5423–5431.

Morley JE, Flood JF, Horowitz M, Morley PM, and Walter MJ (1994) Modulation of food intake by peripherally administered amylin. *Am J Physiol* **267**:R178–R184.

Morley JE, Morley PM, and Flood JF (1993) Anorectic effects of amylin in rats over the life span. *Pharmacol Biochem Behav* **44**:577–580.

Morley JE, Suarez MD, Mattamal M, and Flood JF (1997) Amylin and food intake in mice: effects on motivation to eat and mechanism of action. *Pharmacol Biochem Behav* **56**:123–129.

Moyses C, Kolterman O, and Mant T (1993) Pharmacokinetic and hyperglycaemic effects of the amylin analogue, AC137, in man. *Diabetic Med* **10**:S25.

Muff R, Bühlmann N, Fischer JA, and Born W (1999) An amylin receptor is revealed following co-transfection of a calcitonin receptor with receptor activity modifying proteins-1 or -3. *Endocrinology* **140**:2924–2927.

Müller TD, Sullivan LM, Habegger K, Yi C-X, Kabra D, Grant E, Ottaway N, Krishna R, Holland J, and Hembree J, et al. (2012) Restoration of leptin responsiveness in diet-induced obese mice using an optimized leptin analog in combination with exendin-4 or FGF21. *J Pept Sci* **18**:383–393.

Nakamoto H, Soeda Y, Takami S, Minami M, and Satoh M (2000) Localization of calcitonin receptor mRNA in the mouse brain: coexistence with serotonin transporter mRNA. *Brain Res Mol Brain Res* **76**:93–102.

Nakanome C, Akai H, Hongo M, Imai N, Toyota T, Goto Y, Okuguchi F, and Komatsu K (1983) Disturbances of the alimentary tract motility and hypermotilinemia in the patients with diabetes mellitus. *Tohoku J Exp Med* **139**:205–215.

Nakazato M, Miyazato M, Asai J, Mitsukawa T, Kangawa K, Matsuo H, and Matsukura S (1990) Islet amyloid polypeptide, a novel pancreatic peptide, is a circulating hormone secreted under glucose stimulation. *Biochem Biophys Res Commun* **169**:713–718.

Nauck MA, Heimesaat MM, Behle K, Holst JJ, Nauck MS, Ritzel R, Hüfner M, and Schmiegel WH (2002) Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. *J Clin Endocrinol Metab* **87**:1239–1246.

Nicandro JPA, Ellero C, Pannacciulli N, Kesty NC, Deng W, Weyer C, and Chen HC (2008) AC2307, an amylin mimetic, reduced 24-h food intake in obese subjects (Abstract). *Diabetes* **57** (Suppl 1):A433.

Nyholm B, Møller N, Gravholt CH, Orskov L, Mengel A, Bryan G, Moyses C, Alberti KG, and Schmitz O (1996) Acute effects of the human amylin analog AC137 on basal and insulin-stimulated euglycemic and hypoglycemic fuel metabolism in patients with insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* **81**:1083–1089.

Nyholm B, Orskov L, Hove KY, Gravholt CH, Møller N, Alberti KG, Moyses C, Kolterman O, and Schmitz O (1999) The amylin analog pramlintide improves glycemic control and reduces postprandial glucagon concentrations in patients with type 1 diabetes mellitus. *Metabolism* **48**:935–941.

Ogawa A, Harris V, McCorkle SK, Unger RH, and Luskey KL (1990) Amylin secretion from the rat pancreas and its selective loss after streptozotocin treatment. *J Clin Invest* **85**:973–976.

Olgiati VR, Guidobono F, Netti C, and Pecile A (1983) Localization of calcitonin binding sites in rat central nervous system: evidence of its neuroactivity. *Brain Res* **265**:209–215.

Oliver KR, Kane SA, Salvatore CA, Mallee JJ, Kinsey AM, Koblan KS, Keyvan-Fouladi N, Heavens RP, Wainwright A, and Jacobson M, et al. (2001) Cloning, characterization and central nervous system distribution of receptor activity modifying proteins in the rat. *Eur J Neurosci* **14**:618–628.

Olsson M, Herrington MK, Reidelberger RD, Permert J, Gebre-Medhin S, and Arnelo U (2012) Food intake and meal pattern in IAPP knockout mice with and without infusion of exogenous IAPP. *Scand J Gastroenterol* **47**:191–196.

Osaka T, Tsukamoto A, Koyama Y, and Inoue S (2008) Central and peripheral administration of amylin induces energy expenditure in anesthetized rats. *Peptides* **29**:1028–1035.

Osto M, Wielinga PY, Alder B, Walser N, and Lutz TA (2007) Modulation of the satiating effect of amylin by central ghrelin, leptin and insulin. *Physiol Behav* **91**: 566–572.

Perry KJ, Quiza M, Myers DE, Morfis M, Christopoulos G, and Sexton PM (1997) Characterization of amylin and calcitonin receptor binding in the mouse alpha-thyroid-stimulating hormone thyrotroph cell line. *Endocrinology* **138**:3486–3496.

Pieber TR, Roitelman J, Lee Y, Luskey KL, and Stein DT (1994) Direct plasma radioimmunoassay for rat amylin-(1-37): concentrations with acquired and genetic obesity. *Am J Physiol* **267**:E156–E164.

Plourde V, St-Pierre S, Fournier A, and Taché Y (1993) CGRP 8-37[correction of 8-27] blocks the inhibition of gastric emptying induced by intravenous injection of alpha-CGRP in rats. *Life Sci* **52**:857–862.

Potes CS, Boyle CN, Wookey PJ, Riediger T, and Lutz TA (2012) Involvement of the extracellular signal-regulated kinase 1/2 signaling pathway in amylin's eating inhibitory effect. *Am J Physiol Regul Integr Comp Physiol* **302**:R340–R351.

Potes CS and Lutz TA (2010) Brainstem mechanisms of amylin-induced anorexia. *Physiol Behav* **100**:511–518.

Potes CS, Lutz TA, and Riediger T (2010a) Identification of central projections from amylin-activated neurons to the lateral hypothalamus. *Brain Res* **1334**:31–44.

Potes CS, Riediger T, and Lutz TA (2010b) Amylin induces ERK 1/2 phosphorylation in structures of the AP/NTS-LPB-Ce-BSTL axis. *Appetite* **54**:670.

Potes CS, Turek VF, Cole RL, Vu C, Roland BL, Roth JD, Riediger T, and Lutz TA (2010c) Noradrenergic neurons of the area postrema mediate amylin's hypophagic action. *Am J Physiol Regul Integr Comp Physiol* **299**:R623–R631.

Poyner DR, Sexton PM, Marshall I, Smith DM, Quirion R, Born W, Muff R, Fischer JA, and Foord SM (2002) International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors. *Pharmacol Rev* **54**:233–246.

Qi D, Cai K, Wang O, Li Z, Chen J, Deng B, Qian L, and Le Y (2010) Fatty acids induce amylin expression and secretion by pancreatic β-cells. *Am J Physiol Endocrinol Metab* **298**:E99–E107.

Qi T, Christopoulos G, Bailey RJ, Christopoulos A, Sexton PM, and Hay DL (2008) Identification of N-terminal receptor activity-modifying protein residues important for calcitonin gene-related peptide, adrenomedullin, and amylin receptor function. *Mol Pharmacol* **74**:1059–1071.

Qi T, Dong M, Watkins HA, Wootten D, Miller LJ, and Hay DL (2013) Receptor activity-modifying protein-dependent impairment of calcitonin receptor splice variant Δ(1-47)hCT((a)) function. *Br J Pharmacol* **168**:644–657.

Ravussin E, Smith SR, Mitchell JA, Shringarpure R, Shan K, Maier H, Koda JE, and Weyer C (2009) Enhanced weight loss with pramlintide/metreleptin: an integrated neurohormonal approach to obesity pharmacotherapy. *Obesity (Silver Spring)* **17**:1736–1743.

Reidelberger R, Haver A, Chelikani PK, Apenteng B, Perriotte-Olson C, Anders K, Steenson S, and Blevins JE (2012) Effects of leptin replacement alone and with exendin-4 on food intake and weight regain in weight-reduced diet-induced obese rats. *Am J Physiol Endocrinol Metab* **302**:E1576–E1585.

Reidelberger RD, Arnelo U, Granqvist L, and Permert J (2001) Comparative effects of amylin and cholecystokinin on food intake and gastric emptying in rats. *Am J Physiol Regul Integr Comp Physiol* **280**:R605–R611.

Reidelberger RD, Kelsey L, and Heimann D (2002) Effects of amylin-related peptides on food intake, meal patterns, and gastric emptying in rats. *Am J Physiol Regul Integr Comp Physiol* **282**:R1395–R1404.

Riddle M, Pencek R, Charenkavanich S, Lutz K, Wilhelm K, and Porter L (2009) Randomized comparison of pramlintide or mealtime insulin added to basal insulin treatment for patients with type 2 diabetes. *Diabetes Care* **32**:1577–1582.

Riediger T, Schmid HA, Lutz T, and Simon E (2001) Amylin potently activates AP neurons possibly via formation of the excitatory second messenger cGMP. *Am J Physiol Regul Integr Comp Physiol* **281**:R1833–R1843.

Riediger T, Schmid HA, Lutz TA, and Simon E (2002) Amylin and glucose co-activate area postrema neurons of the rat. *Neurosci Lett* **328**:121–124.
Riediger T, Schmid HA, Young AA, and Simon E (1999) Pharmacological characterisation of amylin-related peptides activating subfornical organ neurones. *Brain Res* **837**:161–168.
Riediger T, Zuend D, Becskei C, and Lutz TA (2004) The anorectic hormone amylin contributes to feeding-related changes of neuronal activity in key structures of the gut-brain axis. *Am J Physiol Regul Integr Comp Physiol* **286**:R114–R122.
Riedy CA, Chavez M, Figlewicz DP, and Woods SC (1995) Central insulin enhances sensitivity to cholecystokinin. *Physiol Behav* **58**:755–760.
Roth CL, Bongiovanni KD, Gohlke B, and Woelfle J (2010) Changes in dynamic insulin and gastrointestinal hormone secretion in obese children. *J Pediatr Endocrinol Metab* **23**:1299–1309.
Roth JD, Coffey T, Jodka CM, Maier H, Athanacio JR, Mack CM, Weyer C, and Parkes DG (2007b) Combination therapy with amylin and peptide YY[3-36] in obese rodents: anorexigenic synergy and weight loss additivity. *Endocrinology* **148**: 6054–6061.
Roth JD, D'Souza L, Griffin PS, Athanacio J, Trevaskis JL, Nazarbaghi R, Jodka C, Athanacio J, Hoyt J, and Forood B, et al. (2012) Interactions of amylinergic and melanocortinergic systems in the control of food intake and body weight in rodents. *Diabetes Obes Metab* **14**:608–615.
Roth JD, Hughes H, Coffey T, Maier H, Trevaskis JL, and Anderson CM (2007a) Effects of prior or concurrent food restriction on amylin-induced changes in body weight and body composition in high-fat-fed female rats. *Am J Physiol Endocrinol Metab* **293**:E1112–E1117.
Roth JD, Hughes H, Kendall E, Baron AD, and Anderson CM (2006) Antiobesity effects of the beta-cell hormone amylin in diet-induced obese rats: effects on food intake, body weight, composition, energy expenditure, and gene expression. *Endocrinology* **147**:5855–5864.
Roth JD, Maier H, Chen S, and Roland BL (2009) Implications of amylin receptor agonism: integrated neurohormonal mechanisms and therapeutic applications. *Arch Neurol* **66**:306–310.
Roth JD, Roland BL, Cole RL, Trevaskis JL, Weyer C, Koda JE, Anderson CM, Parkes DG, and Baron AD (2008a) Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. *Proc Natl Acad Sci USA* **105**:7257–7262.
Roth JD, Soares CJ, Ghosh SS, and Parkes DG (2008b) Amylin-based pharmacotherapy – past, present & future. *Immunol Endocr Metab Agents Med Chem* **8**: 317–324.
Roth JD, Trevaskis JL, Wilson J, Lei C, Athanacio J, Mack C, Kesty NC, Coffey T, Weyer C, and Parkes DG (2008c) Antiobesity effects of the beta-cell hormone amylin in combination with phentermine or sibutramine in diet-induced obese rats. *Int J Obes (Lond)* **32**:1201–1210.
Rowland NE, Crews EC, and Gentry RM (1997) Comparison of Fos induced in rat brain by GLP-1 and amylin. *Regul Pept* **71**:171–174.
Rushing PA, Hagan MM, Seeley RJ, Lutz TA, D'Alessio DA, Air EL, and Woods SC (2001) Inhibition of central amylin signaling increases food intake and body adiposity in rats. *Endocrinology* **142**:5035.
Rushing PA, Hagan MM, Seeley RJ, Lutz TA, and Woods SC (2000) Amylin: a novel action in the brain to reduce body weight. *Endocrinology* **141**:850–853.
Sabharwal R, Zhang Z, Lu Y, Abboud FM, Russo AF, and Chapleau MW (2010) Receptor activity-modifying protein 1 increases baroreflex sensitivity and attenuates Angiotensin-induced hypertension. *Hypertension* **55**:627–635.
Saetrum Opgaard O, de Vries R, Tom B, Edvinsson L, and Saxena PR (1999) Positive inotropy of calcitonin gene-related peptide and amylin on porcine isolated myocardium. *Eur J Pharmacol* **385**:147–154.
Samsom M, Szarka LA, Camilleri M, Vella A, Zinsmeister AR, and Rizza RA (2000) Pramlintide, an amylin analog, selectively delays gastric emptying: potential role of vagal inhibition. *Am J Physiol Gastrointest Liver Physiol* **278**:G946–G951.
Schorr AB and Ofan R (2012) Simultaneous use of two external subcutaneous pumps delivering insulin and SYMLIN: use of a double-pump system. *J Diabetes Sci Tech* **6**:1507–1508.
Sexton PM, Findlay DM, and Martin TJ (1999) Calcitonin. *Curr Med Chem* **6**: 1067–1093.
Sexton PM, McKenzie JS, and Mendelsohn FA (1988) Evidence for a new subclass of calcitonin/ calcitonin gene-related peptide binding site in rat brain. *Neurochem Int* **12**:323–335.
Sexton PM, Paxinos G, Huang XF, and Mendelsohn FA (1994a) In vitro autoradiographic localization of calcitonin binding sites in human medulla oblongata. *J Comp Neurol* **341**:449–463.
Sexton PM, Paxinos G, Kenney MA, Wookey PJ, and Beaumont K (1994b) In vitro autoradiographic localization of amylin binding sites in rat brain. *Neuroscience* **62**: 553–567.
Sibilia V, Pagani F, Lattuada N, Rapetti D, Guidobono F, and Netti C (2000) Amylin compared with calcitonin: competitive binding studies in rat brain and antinociceptive activity. *Brain Res* **854**:79–84.
Silvestre RA, Rodríguez-Gallardo J, Jodka C, Parkes DG, Pittner RA, Young AA, and Marco J (2001) Selective amylin inhibition of the glucagon response to arginine is extrinsic to the pancreas. *Am J Physiol Endocrinol Metab* **280**:E443–E449.
Singh-Franco D, Perez A, and Harrington C (2011) The effect of pramlintide acetate on glycemic control and weight in patients with type 2 diabetes mellitus and in obese patients without diabetes: a systematic review and meta-analysis. *Diabetes Obes Metab* **13**:169–180.
Skofitsch G, Wimalawansa SJ, Jacobowitz DM, and Gubisch W (1995) Comparative immunohistochemical distribution of amylin-like and calcitonin gene related peptide like immunoreactivity in the rat central nervous system. *Can J Physiol Pharmacol* **73**:945–956.
Smeltzer M, Scott K, Melhorn S, Krause E, and Sakai R (2012) Amylin blunts hyperphagia and reduces weight and fat gain during recovery in socially stressed rats. *Am J Physiol Regul Integr Comp Physiol* **303**:R676–R682.
Smith SR, Aronne LJ, Burns CM, Kesty NC, Halseth AE, and Weyer C (2008) Sustained weight loss following 12-month pramlintide treatment as an adjunct to lifestyle intervention in obesity. *Diabetes Care* **31**:1816–1823.
Smith SR, Blundell JE, Burns C, Ellero C, Schroeder BE, Kesty NC, Chen KS, Halseth AE, Lush CW, and Weyer C (2007) Pramlintide treatment reduces 24-h caloric intake and meal sizes and improves control of eating in obese subjects: a 6-wk translational research study. *Am J Physiol Endocrinol Metab* **293**:E620–E627.
Stachniak TJE and Krukoff TL (2003) Receptor activity modifying protein 2 distribution in the rat central nervous system and regulation by changes in blood pressure. *J Neuroendocrinol* **15**:840–850.
Surina-Baumgartner DM, Langhans W, and Geary N (1995) Hepatic portal insulin antibody infusion increases, but insulin does not alter, spontaneous meal size in rats. *Am J Physiol* **269**:R978–R982.
Szabó ÉR, Cservenák M, and Dobolyi A (2012) Amylin is a novel neuropeptide with potential maternal functions in the rat. *FASEB J* **26**:272–281.
Tam CW, Husmann K, Clark NC, Clark JE, Lazar Z, Ittner LM, Götz J, Douglas G, Grant AD, and Sugden D, et al. (2006) Enhanced vascular responses to adrenomedullin in mice overexpressing receptor-activity-modifying protein 2. *Circ Res* **98**: 262–270.
Tashjian AH Jr, Wright DR, Ivey JL, and Pont A (1978) Calcitonin binding sites in bone: relationships to biological response and "escape." *Recent Prog Horm Res* **34**: 285–334.
Tilakaratne N, Christopoulos G, Zumpe ET, Foord SM, and Sexton PM (2000) Amylin receptor phenotypes derived from human calcitonin receptor/RAMP coexpression exhibit pharmacological differences dependent on receptor isoform and host cell environment. *J Pharmacol Exp Ther* **294**:61–72.
Tingstedt JE, Edlund H, Madsen OD, and Larsson LI (1999) Gastric amylin expression. Cellular identity and lack of requirement for the homeobox protein PDX-1. A study in normal and PDX-1-deficient animals with a cautionary note on antiserum evaluation. *J Histochem Cytochem* **47**:973–980.
Tomabechi Y, Krippner G, Rendle PM, Squire MA, and Fairbanks AJ (2013) Glycosylation of pramlintide: synthetic glycopeptides that display in vitro and in vivo activities as amylin receptor agonists. *Chemistry* **19**:15084–15088.
Trevaskis JL, Coffey T, Cole R, Lei C, Wittmer C, Walsh B, Weyer C, Koda J, Baron AD, and Parkes DG, et al. (2008) Amylin-mediated restoration of leptin responsiveness in diet-induced obesity: magnitude and mechanisms. *Endocrinology* **149**:5679–5687.
Trevaskis JL, Lei C, Koda JE, Weyer C, Parkes DG, and Roth JD (2010a) Interaction of leptin and amylin in the long-term maintenance of weight loss in diet-induced obese rats. *Obesity (Silver Spring)* **18**:21–26.
Trevaskis JL, Parkes DG, and Roth JD (2010b) Insights into amylin-leptin synergy. *Trends Endocrinol Metab* **21**:473–479.
Trevaskis JL, Turek VF, Wittmer C, Griffin PS, Wilson JK, Reynolds JM, Zhao Y, Mack CM, Parkes DG, and Roth JD (2010c) Enhanced amylin-mediated body weight loss in estradiol-deficient diet-induced obese rats. *Endocrinology* **151**: 5657–5668.
Turek VF, Trevaskis JL, Levin BE, Dunn-Meynell AA, Irani B, Gu G, Wittmer C, Griffin PS, Vu C, and Parkes DG, et al. (2010) Mechanisms of amylin/leptin synergy in rodent models. *Endocrinology* **151**:143–152.
Turner AG, Tjahyono F, Chiu WSM, Skinner J, Sawyer R, Moore AJ, Morris HA, Findlay DM, Zajac JD, and Davey RA (2011) The role of the calcitonin receptor in protecting against induced hypercalcemia is mediated via its actions in osteoclasts to inhibit bone resorption. *Bone* **48**:354–361.
Udawela M, Christopoulos G, Morfis M, Christopoulos A, Ye S, Tilakaratne N, and Sexton PM (2006a) A critical role for the short intracellular C terminus in receptor activity-modifying protein function. *Mol Pharmacol* **70**:1750–1760.
Udawela M, Christopoulos G, Tilakaratne N, Christopoulos A, Albiston A, and Sexton PM (2006b) Distinct receptor activity-modifying protein domains differentially modulate interaction with calcitonin receptors. *Mol Pharmacol* **69**: 1984–1989.
Udawela M, Christopoulos G, Morfis M, Tilakaratne N, Christopoulos A, and Sexton PM (2008) The effects of C-terminal truncation of receptor activity modifying proteins on the induction of amylin receptor phenotype from human CTb receptors. *Regul Pept* **145**:65–71.
Ueda T, Ugawa S, Saishin Y, and Shimada S (2001) Expression of receptor-activity modifying protein (RAMP) mRNAs in the mouse brain. *Brain Res Mol Brain Res* **93**:36–45.
Vine W, Smith P, LaChappell R, Blase E, Lumpkin R, and Young A (1998a) Nephrectomy decreases amylin and pramlintide clearance in rats. *Horm Metab Res* **30**:514–517.
Vine W, Smith P, LaChappell R, Blase E, and Young A (1998b) Effects of rat amylin on renal function in the rat. *Horm Metab Res* **30**:518–522.
Visa M, Alcarraz-Vizan G, Montane J, Cadavez L, Castano C, Villanueva-Penacarrillo ML, Servitja JM, and Novials A (2015) Islet amyloid polypeptide exerts a novel autocrine action in β-cell signaling and proliferation. *FASEB J* DOI: 10.1096/fj.15-270553 [published ahead of print]
Walker CS, Eftekhari S, Bower RL, Wilderman A, Insel PA, Edvinsson L, Waldvogel HJ, Jamaluddin MA, Russo AF, and Hay DL (2015) A second trigeminal CGRP receptor: function and expression of the AMY1 receptor. *Ann Clin Transl Neurol* DOI: 10.1002/acn3.197 [published ahead of print]
Walker CS, Li X, Whiting L, Glyn-Jones S, Zhang S, Hickey AJ, Sewell MA, Ruggiero K, Phillips ARJ, and Kraegen EW, et al. (2010) Mice lacking the neuropeptide α-calcitonin gene-related peptide are protected against diet-induced obesity. *Endocrinology* **151**:4257–4269.
Wauman J and Tavernier J (2011) Leptin receptor signaling: pathways to leptin resistance. *Front Biosci (Landmark Ed)* **16**:2771–2793.
Weinzimer SA, Sherr JL, Cengiz E, Kim G, Ruiz JL, Carria L, Voskanyan G, Roy A, and Tamborlane WV (2012) Effect of pramlintide on prandial glycemic excursions during closed-loop control in adolescents and young adults with type 1 diabetes. *Diabetes Care* **35**:1994–1999.

West DB, Fey D, and Woods SC (1984) Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. *Am J Physiol* **246**:R776–R787.
Westermark P, Andersson A, and Westermark GT (2011) Islet amyloid polypeptide, islet amyloid, and diabetes mellitus. *Physiol Rev* **91**:795–826.
Westermark P, Wernstedt C, O'Brien TD, Hayden DW, and Johnson KH (1987a) Islet amyloid in type 2 human diabetes mellitus and adult diabetic cats contains a novel putative polypeptide hormone. *Am J Pathol* **127**:414–417.
Westermark P, Wernstedt C, Wilander E, Hayden DW, O'Brien TD, and Johnson KH (1987b) Amyloid fibrils in human insulinoma and islets of Langerhans of the diabetic cat are derived from a neuropeptide-like protein also present in normal islet cells. *Proc Natl Acad Sci USA* **84**:3881–3885.
Westermark P, Wernstedt C, Wilander E, and Sletten K (1986) A novel peptide in the calcitonin gene related peptide family as an amyloid fibril protein in the endocrine pancreas. *Biochem Biophys Res Commun* **140**:827–831.
Weyer C, Fineman MS, Strobel S, Shen L, Data J, Kolterman OG, and Sylvestri MF (2005) Properties of pramlintide and insulin upon mixing. *Am J Health Syst Pharm* **62**:816–822.
Weyer C, Maggs DG, Young AA, and Kolterman OG (2001) Amylin replacement with pramlintide as an adjunct to insulin therapy in type 1 and type 2 diabetes mellitus: a physiological approach toward improved metabolic control. *Curr Pharm Des* **7**: 1353–1373.
Wickbom J, Herrington MK, Permert J, Jansson A, and Arnelo U (2008) Gastric emptying in response to IAPP and CCK in rats with subdiaphragmatic afferent vagotomy. *Regul Pept* **148**:21–25.
Wielinga PY, Alder B, and Lutz TA (2007) The acute effect of amylin and salmon calcitonin on energy expenditure. *Physiol Behav* **91**:212–217.
Wielinga PY, Löwenstein C, Alder B, and Lutz TA (2008) Effect of peripheral and central amylin on energy expenditure and body temperature. *Appetite* **91**:409.
Wielinga PY, Löwenstein C, Muff S, Munz M, Woods SC, and Lutz TA (2010) Central amylin acts as an adiposity signal to control body weight and energy expenditure. *Physiol Behav* **101**:45–52.
Wong WP, Scott DW, Chuang CL, Zhang S, Liu H, Ferreira A, Saafi EL, Choong YS, and Cooper GJ (2008) Spontaneous diabetes in hemizygous human amylin transgenic mice that developed neither islet amyloid nor peripheral insulin resistance. *Diabetes* **57**:2737–2744.
Woods SC (2005) Signals that influence food intake and body weight. *Physiol Behav* **86**:709–716.
Wookey PJ, Cao Z, van Geenen RC, Voskuil M, Darby IA, Komers R, and Cooper ME (1997) Increased density of renal amylin binding sites in experimental hypertension. *Hypertension* **30**:455–460.
Wookey PJ and Cooper ME (1998) Amylin: physiological roles in the kidney and a hypothesis for its role in hypertension. *Clin Exp Pharmacol Physiol* **25**:653–660.
Wookey PJ, Tikellis C, Nobes M, Casley D, Cooper ME, and Darby IA (1998) Amylin as a growth factor during fetal and postnatal development of the rat kidney. *Kidney Int* **53**:25–30.
Yang Y and Song W (2013) Molecular links between Alzheimer's disease and diabetes mellitus. *Neuroscience* **250**:140–150.
Young A (1997) Role of amylin in nutrient intake - animal studies. *Diabet Med* **14** (Suppl 2):S14–S18.
Young A (2005a) Cardiovascular effects. *Adv Pharmacol* **52**:239–250.
Young A (2005b) Inhibition of gastric emptying. *Adv Pharmacol* **52**:99–121.
Young A (2005c) Inhibition of glucagon secretion. *Adv Pharmacol* **52**:151–171.
Young A (2005d) Tissue expression and secretion of amylin. *Adv Pharmacol* **52**: 19–45.
Young A and Denaro M (1998) Roles of amylin in diabetes and in regulation of nutrient load. *Nutrition* **14**:524–527.
Young A, Kolterman O, and Hall J (1999) Amylin innocent in essential hypertension? *Diabetologia* **42**:1029.
Young AA (2012) Brainstem sensing of meal-related signals in energy homeostasis. *Neuropharmacology* **63**:31–45.
Young AA, Gedulin B, Vine W, Percy A, and Rink TJ (1995) Gastric emptying is accelerated in diabetic BB rats and is slowed by subcutaneous injections of amylin. *Diabetologia* **38**:642–648.
Young AA, Gedulin BR, and Rink TJ (1996a) Dose-responses for the slowing of gastric emptying in a rodent model by glucagon-like peptide (7-36) NH2, amylin, cholecystokinin, and other possible regulators of nutrient uptake. *Metabolism* **45**: 1–3.
Young AA, Vine W, Gedulin BR, Pittner R, Janes S, Gaeta LSL, Percy A, Moore CX, Koda JE, and Rink TJ, et al. (1996b) Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. *Drug Dev Res* **37**:231–248.
Young AA, Wang MW, and Cooper GJ (1991) Amylin injection causes elevated plasma lactate and glucose in the rat. *FEBS Lett* **291**:101–104.
Younk LM, Mikeladze M, and Davis SN (2011) Pramlintide and the treatment of diabetes: a review of the data since its introduction. *Expert Opin Pharmacother* **12**: 1439–1451.
Zaki M, Koduru S, McCuen R, Vuyyuru L, and Schubert ML (2002) Amylin, released from the gastric fundus, stimulates somatostatin and thus inhibits histamine and acid secretion in mice. *Gastroenterology* **123**:247–255.
Zhang Y and Scarpace PJ (2006) The role of leptin in leptin resistance and obesity. *Physiol Behav* **88**:249–256.
Zhang Z, Liu X, Morgan DA, Kuburas A, Thedens DR, Russo AF, and Rahmouni K (2011) Neuronal receptor activity-modifying protein 1 promotes energy expenditure in mice. *Diabetes* **60**:1063–1071.
Zhang Z, Winborn CS, Marquez de Prado B, and Russo AF (2007) Sensitization of calcitonin gene-related peptide receptors by receptor activity-modifying protein-1 in the trigeminal ganglion. *J Neurosci* **27**:2693–2703.
Zhou Y and Rui L (2013) Leptin signaling and leptin resistance. *Fr Medecine* **7**:207–222.
Zhu H, Wang X, Wallack M, Li H, Carreras I, Dedeoglu A, Hur JY, Zheng H, Fine R, and Mwamburi M, et al. (2015) Intraperitoneal injection of the pancreatic peptide amylin potently reduces behavioral impairment and brain amyloid pathology in murine models of Alzheimer's disease. *Mol Psychiatry* **20**:252–262.
Züger D, Forster K, Lutz TA, and Riediger T (2013) Amylin and GLP-1 target different populations of area postrema neurons that are both modulated by nutrient stimuli. *Physiol Behav* **112–113**:61–69.